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SYMPOSIUM ON THE ANALYSIS OF CARCINOGENIC AIR POLLUTANTS HELD AT CINCINNATI, OHIO ON AUGUST 29, 1961. VOLUME I

E. Sawicki, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

29 August 1961

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VOLUME I

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SYMPOSIUM

on

THE ANALYSIS OF CARCINOGENIC AIR POLLUTANTS

- - -

August 29, 1961

at

Cincinnati, Ohio

DR. E. SAWICKI, CHAIRMAN

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Sponsored by the

LABORATORY OF ENGINEERING AND PHYSICAL SCIENCES

of the

DIVISION OF AIR POLLUTION

- - -

ROBERT A. TAFT

SANITARY ENGINEERING CENTER

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- - -

PERSONS ATTENDING

Name	Affiliation	Location
Dr. G. M. Badger	University of Adelaide	Adelaide, S. Australia
Mr. C. R. Begeman	General Motors	Warren, Michigan
Dr. W. F. Biller	Esso Research	Linden, New Jersey
Mr. J. M. Colucci	General Motors	Warren, Michigan
Dr. N. Carugno	e Laboratoria di Biochimica	Rome, Italy
Mr. B. T. Commins	Dunn Laboratories	London E.C.1, England
Dr. F. Dupire X	Laboratoire de Recherches	Tertre, Belgium
Dr. H. L. Falk	University of S. California	Los Angeles 33, Calif.
Mr. F. L. Gager, Jr.	Philip Morris	Richmond, Virginia
Dr. H. Helwig	California Dept. Health	Berkeley, California
Dr. D. Hoffmann	Sloan Kettering Institute	New York, N. Y.
Mrs. Hoffmann	Sloan Kettering Institute	New York, N. Y.
Mr. H. Howerton	American Instrument Company	Silver Spring, Maryland
Dr. M. Katz	Dept. Nat'l Health & Welfare	Ottawa, Canada
Dr. M. Kuratsune	Kyushu University	Fukuoka, Japan
Dr. A. J. Lindsey	Sir John Cass College	London E.C.3, England
Dr. M. J. Lyons	Royal Beatson Memorial Hosp.	Glasgow, Scotland
Dr. W. W. Payne	National Cancer Institute	Bethesda, Maryland
Dr. R. C. Sahni +	New York University	New York, N. Y.
Dr. J. F. Thomas	University of California	Berkeley, California
Dr. R. S. Tipson	Nat'l Bu. of Standards	Washington, D. C.
Dr. B. L. Van Duuren	Bellevue Medical Center	New York, N. Y.
Mr. C. D. Yaffe	Coordinator Schenck Act	Washington, D. C.

ROBERT A. TAFT SANITARY ENGINEERING CENTER PERSONNEL

Dr. A. P. Altshuller
Mr. D. F. Bender
Mr. R. H. Burttschell
Mr. F. T. Fox
Mr. T. R. Hauser
Mr. D. R. Johnston
Mr. S. L. Kopczynski
Dr. J. H. Ludwig
Mr. J. E. Meeker
Dr. J. Miranda
Mr. J. D. Pfaff
Dr. E. Sawicki
Mr. S. F. Sleva
Mr. T. W. Stanley
Mr. A. C. Stern
Mr. E. C. Tabor
Mr. D. J. Von Lehmden
Mr. R. M. Wilson, Jr.
Mr. W. W. Stalker

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PROCEEDINGS

CHAIRMAN SAWICKI: We welcome our foreign visitors, the speakers and all other participants to this meeting at Robert A. Taft Sanitary Engineering Center.

I would like to introduce the first speaker. He has been the driving force in initiating this meeting and has been a powerful factor in placing Government research in air pollution on a firm foundation. He is the Chief of the Laboratory of Engineering and Physical Sciences which is sponsoring this meeting. Mr. Arthur Stern.

MR. STERN: Well, I am going to repeat the welcome that Dr. Sawicki gave you, only this time it's on behalf of both the Division of Air Pollution of the Public Health Service and the Sanitary Engineering Center, the building in which this meeting is being held.

Now, the reason we are holding this symposium is entirely a selfish one on our part. Now, we wanted to tap the best brains in the world working on the problem to improve our own ability to educate and measure carcinogenic air pollutants. We regret that our scientific colleagues from U.S.S.R. whom we also invited to this meeting were unable to attend. This was almost the sole exception of the folks whom we invited here who had to decline so that we feel that we are proud to have assembled such a distinguished group of scientists working in this field and hope that the interchange of information will be beneficial to all of us.

Now, let me first explain something to you about the organization in the United States responsible for research in air pollution. The department in our Federal Government that has this responsibility is the Department of Health, Education and Welfare. It's headquarters, of course, are in Washington, D. C. and our Secretary is Abraham Ribicoff, the Secretary of the Department of Health, Education and Welfare.

The particular organization within the Department of Health, Education and Welfare responsible for research in public health in the United States Government is the U. S. Public Health Service. The Public Health Service is under the direction of a Surgeon General, Dr. Luther Terry and the headquarters are also in Washington, D. C.

Now, as a part of the Public Health Service we have one bureau responsible for doing the kinds of research of need to the various states and communities and the population as a whole and this goes by the name of the Bureau of State Services. That is under the supervision of Dr. Theodore Bower and that is also headquartered in Washington.

I am taking you through

all these intricacies of our internal organization on the theory that some of you don't know where you are, those of you who came from abroad and I am trying to orient you in our scheme of things as to where you are organizationally and then I will tell you where you are geographically.

Last September within the Bureau there was set up a Division of Air Pollution. I will take you back about four or five or six years and just quickly take you through the growth of air pollution research within the United States Government.

The first formal research activity in air polution -- when I say formal I mean recognised as you will recognise the word formal by budgetary appropriation. We don't count research unless somebody pays for it. So that although there had been some effort in air pollution study prior to 1954 it was done by diverting other efforts to meet special circumstances.

For instance, the Public Health Service did respond to the disaster in Donora, Pennsylvania by diverting some of the activities of our Division of Occupational Health and mustering a team to go into the field, but this was done at the expense of other activities and as soon as the report on the disaster at Donora was completed the team was disbanded and no further formal activity was undertaken.

But in late 1953 the Sanitary Engineering Center was the recipient of a contract from the Army Chemical Corps to determine the protein concentration of the atmosphere in a number of parts of the United States, and the folks here who at that time set up the sampling network throughout the country to get the samples from which to determine the protein content recognized that the samples were of more use than merely the determination of proteins and they made simple steps into the measurement of several other measurable quantities within the filter samples which were set out. This led to the initial appropriation for air pollution work in 1954 and this was for an intensification and a continuation of this effort which was soon named the National Air Sampling Network.

In 1955 air pollution was recognized as a separate research entity here at Sanitary Engineering Center and an initial organization was created which grew over the years from about two or three people in 1955 and an appropriation of around $80,000 a year to our present overall air pollution organization budget. Now the present budget level of air pollution research, technical assistance and training in the Federal establishment is $8,600,000 of which about eighty percent goes into research efforts.

Now, our research efforts

are divided about equally into three parts. About one-third is research grant activity where funds are made available to individual researchers in universities to do work on projects of their own choosing but, of course, these projects are carefully screened to determine whether they have adequate merit and adquate priority to receive funds in order that the researchers may proceed.

And, incidentally, there is no national limitation on research grants. We have provided research grant funds to researchers in countries other than the United States where the overall benefit to science will be the ultimate result. And if any of you are interested in what steps are necessary to apply for research grants we will be glad to give you this information. I am not trying to plug grants, I am just trying to give you a bit of background and tell you what the story is. So this represents about one-third of the air pollution activity.

Another third represents essentially contract activity, the largest part of which is with other Federal agencies. We have fairly substantial contractual arrangements for research with the United States Bureau of Standards, the United States Bureau of Mines, the United States Weather Bureau, the Department of Agriculture, the Tennessee Valley Authority and the Library of Congress, to mention the principal ones with whom we have this type of relationship. And in addition there are substantial contracts with university hospital groups and universities to do primarily epidemiological type of studies.

And then the final third of our overall activity is that which we do directly with our own staff, our own equipment and our own funds. And our direct activity is divided within the Division of Air Pollution into several operating branches. The one is a very small operating branch, it's a one responsible for research grants and for training grants. This is just a group of about three or four people located in Washington.

Then the other four groups, one of which is a technical assistance group. This has its headquarters here in Cincinnati and is set up to translate the results of research and prior knowledge into the kind of information and assistance needed by operating air pollution control agencies in the states and through them in the many communities of the United States.

Then the remaining three groups are research groups. There is a field studies group primarily concerned with the type of study where we will set up a study in a community and stay for a year or two with both aerometric or air measuring components and medical epidemiological measurements to get the relationship -- to establish the relationship in that community between what we can measure and find out about the pollutantsin the atmosphere and what we can find out about the incidence of various diseases in the affected population.

Now, this, of course, is not as you all recognize a simple thing of cause and effect. None of us have ever been able to really do a good job of cause and effect. All we establish, as you know, are statistical relationships, collations, probabilities and good guesses, and bad guesses too.

So this represents essentially the type of activity of our field studies group.

And then the two remaining groups are those which are primarily laboratory oriented and the one is our Laboratory of Medical and Biological Sciences which has its principal laboratory not at this building but at an annex of this building which is about twelve miles away from here in Cincinnati. We know it by the name of the Toms River Building. It has been just recently acquired within the past several months. The group is just getting set up there. You are welcome to arrange to visit it during your stay here. I think you will find the activity over there will be of interest but I am not trying to oversell what you would see at Toms River Building at this time because the groups are just in the stage of moving into it, but this will be in the future -- at least for the next five years -- the principal research center for animal experimentation on the effects of air pollutions on experimental animals, and this will be the major role of that laboratory. It will be a very large establishment, with large animal colony facilities, facilities for generating pollutants of various kinds and exposing animals and will, of course, have all this necessary supporting laboratories, chemical and physical analysis to do the necessary biochemistry and other types of work necessary to support a medical and biological study.

We have one small component of that group on our campus here and that is across the road from here. We have the greenhouse and the laboratory associated with the agricultural research component of the Laboratory of Medical and Biological Sciences. This is over here primarily because the principal use of the greenhouse is to grow plants which are exposed to pollutants generated in this building as a part of the activity of the final group that I am going to mention and that's the group who are your immediate host here, the Laboratory of Engineering and Physical Sciences, and we are your hosts here and hope we will be able to make your stay pleasant while you are here.

Now, the Laboratory of Engineering and Physical Sciences, your host, is again subdivided into several operating groups. The one that I mentioned originally as being the precursor of all of our air pollution activity here in the Public Health Service, the National Air Sampling Network. This is one of the operating sections of the Laboratory of Engineering and Physical Sciences and it actually now operates a multiplicity of National Air Sampling Networks. We are still continuing in excess of 200 cities in the United States and a number of non-urban locations the type of measurement that we initiated in late 1953 and intensified in 1954. That is the type of sampling based upon twenty-four hour samples through an eight by ten glass fiber filter. These samples are taken on essentially a biweekly basis in each of the cities where the samples are taken and they are returned to this laboratory for a battery of tests and analyses. Actually the total number of types of analyses that we will run on the samples which are most intensively analyzed will run close to thirty, whereas some of the samples we give a much more cursory analysis to, but by and large the determination as to the intensity of analysis depends on the type of interest we have in research at the particular time. The samples remain available for further analysis and we have something in excess of 20,000 of these samples available as a library for any type of analysis we choose to make. Although a large part of this has already been collated and published and we are continually in the process of assembling and collating and publishing this type of data.

The second of the National networks is a gas sampling network in 50 cities in the United States where we are measuring again on a twenty=four hour average basis and on essentially a biweekly basis sulphur dioxide, oxides of nitrogen and piloting several other types of measurements. We have not had a satisfactory method for oxidant that can be used to send samples back and forth to the center for analysis.

We are just in the process of setting up in eight cities in the United States a continuous gas sampling network which will give us continuously on punch paper tape every five minutes an analysis for seven gases in the air, carbon monoxide, sulphur dioxide, hydrocarbon, NO, NO^2, ozone and oxidant. That adds up to seven. Those are the gases which will be measured in this manner and analysed by computer and in addition at these cities parallel measurements will be made of particulate rainfall and other sources of samples of our interest.

The fourth of the networks for which the National Air Sampling Network is responsible is a precipitation network where we are concerned in some thirty locations throughout the United States in the measurement of rainfall and the measurable components in rainfall.

And then a fifth one with which this group has only partial identification -- the principal group being responsible is our meteorological group -- is a turbidity trepidity measuring network based on viewing the sun at 0.5 wave length and determining the intensity of sunlight coming through as it is diminished by particulate between the observer and the sun. This is a very sensitive measure and we find that we can get information almost as good in terms of total concentration of particulate in the atmosphere as from direct filter measurements. And this is going on in a number of locations in the country to get this on a firmer basis.

So those are the prime functions of our National Air Sampling Network. I mentioned that one of these networks is the prime function of our meteorology group. This is a group of meteorologists who are assigned to us by the United States Weather Bureau and are concerned not only with this type of a trepidity measuring network but also with all of the diffusion and dissemination of pollution problems that we all are familair with. Their role is to explain how pollution gets from its source to the receptor and give us mathematical models for that type of a movement. We are more concerned here with the multiplesource problem than with the single-source problem, although we are not immune to some interest in what happens from the emission from a single stack.

The third of our operating sections is an instrumentation section concerned with the development of all of the kinds of instruments needed by all of the various interests in air pollution, both laboratory and field.

The fourth group is our Engineering Research and Development group concerned primarily with source problems. What is it that comes out of a source of pollution and how do we measure it, how do we control it. Because of the prime interest in the United States at this time in automobile exhaust as a source of pollution, this interest being particularly sponsored by our Congress who have appropriated specific funds for that type of research, our main interest in the Engineering Research and Development group at this time is research in the role of the automobile in the air pollution problem and here we have a pilot plant which generates pollutants from automobiles, irradiates it in a chamber and determines the effect of this on plants, animals, bacteria, the effect on visibility reduction and the chemical analysis.

And then, finally, the group here which is your direct sponsor, the Chemical Research and Development group, which as it names implies is concerned with research into chemical methodology and the chemistry of air pollution, photochemistry of air pollution, chemistry of polynuclear hydrocarbons and so forth.

Now, just a moment about the Sanitary Engineering Center. The Sanitary Engineering Center is this building and all of the related buildings here in Cincinnati and it houses not only the research activities of the Division of Air Pollution but also those in the field of Water Supply and Water Pollution Control, Radiological Health, Milk and Food Research and we have a training activity here concerned with all of these activities.

Now, the question is frequently asked, why is all this in Cincinnati? I know that certainly to people both in the United States and abroad Cincinnati is somewheres in the Midwest where there are Indians behind each rock. If any of you finds an Indian, please let me know. We want to get an authentic Indian picture if we can here in Cincinnati. But we haven't seen an Indian here for quite awhile. The reason that the center is here is that the initial research in Water Supply and Water Pollution back in 1913 was on the Ohio River and it was done from a house-boat and that house-boat anchored eventually here at Cincinnati. The people left the boat, came on land the same way that life started on land originally when the marine creatures came on land. A center was established here first in downtown Cincinnati. In 1953 construction started on this building and the center started here. We soon outgrew this building, started putting temporary buildings across the road and just this past year we acquired another building about half this size twelve miles from here and we are still crowded for space.

Now, just by way of conclusion so I can be through at 10:00 o'clock as your program states, let me just have a moment's discussion of the physical arrangements for the meeting. There will be coffee available at recess time. We will have luncheon available downstairs in the cafeteria. You will have more details about a dinner arranged for Wednesday evening of this week at the Hotel Metropole. For those of you who get completely bored with proceedings there is a library down the hall on the first floor where you will be welcome and nobody will ask you whether you are a refugee from here or not. And if any of you feel that you are constrained to stay over for an extra day to either have discussions with members of our staff or to have a guided tour of not only the air pollution facillities in Cincinnati, but if you are interested, those in Water Pollution, Water Supply, Radiological Health, Milk and Food and training, this we would be glad to arrange either on late Thursday, although maybe your discussions may take you through till too late Thursday for the purpose, or anytime on Friday if you wish.

If any of you should get lost in the building and on the fourth floor and you are looking again for a refuge, the library is not there, you are welcome to drop into my office there and sit down for a chat and we can discuss why it is that you are not down here and why you are up there. Maybe I should be here and I'll have to explain to you why I'm up there and not down here. I am a little bit confused in this whole situation.

Now, before turning the meeting back to Dr. Sawicki, I'd like to introduce two folks whom I think you should know here. One is the Deputy Chief of the Laboratory of Engineering and Physical Sciences, Dr. John Ludwig, and the other is the Chief of the Chemical Research and Development Section, the group with which Dr. Sawicki is associated, Dr. A. P. Altshuller.

And still lacking three minutes, I will turn the meeting back to Dr. Sawicki. Either that or do you want me to do a song and dance for three minutes? Thank you very much.

DR. SAWICKI: Well, I will have to make some announcements and a few other things so these three minutes will come in handy. We hope that a little sandpaper will be applied constructively to the improvement of the present methodology. We hope that the speakers will set the tuning forks in many of our minds vibrating, and finally, we hope that the small Niagara of knowledge pouring out in this room and whatever else the participants meet at this symposium may help initiate further investigations in the Air Pollution field.

Now, after each talk we will have a ten to fifteen-minute period for questions and comments. If the speaker needs more time he can take a little more time. There is no necessity to hurry any talk. We have attempted to provide ample time for each speaker. The person wishing to ask a question or make a comment should first of all clearly identify himself by name, place of employment and city. This will help us in assembling the proceedings.

Some of our people who pass through downtown Cincinnati on their way home from work have volunteered their automotive services. Consequently, we have private transportation for the speakers to downtown Cincinnati leaving this building at about 4:30 p. m. Tuesday, Wednesday and Thursday. We also have transportation to the Hotel Alms and Williams YMCA area. If any speaker wishes this service he should contact me. We have no service for the morning, that you have to provide for yourself. We might even be able to help some of the other participants if they want to leave about that time.

Now, in the program bulletin we have an extra sheet and it may be that some of the speakers who have obtained the program bulletin previously do not have this extra sheet. In this sheet we point out the time for the cocktail hour, the Wednesday night dinner and also a change in the program Thursday afternoon. If any participant runs into any difficulty or has any questions about transportation or some other meeting factor, you can contact me or Dr. Altshuller or anybody else that's going to do this meeting and we'll see what we can do to help you.

Now, we have frequent recesses so that the participants can get acquainted and informally talk shop if they want to. And they tell me that for the participants for this symposium the cafeteria will be open at 12:45, I believe.

Now, a further thing, the two times two slide projector, if you have two times two slides, is automatic and we have a little gadget here where you just push a button and change the slide. The first slide will, of course, be set in there.

Now, Tom Hauser had some other comments. This microphone here we can put around your neck so if you are in the habit of walking back and forth, if you want to get a little exercise while you are talking, then I will put this around your neck, not too tight. Now, the table microphones, we have them set up and we are recording all this so the people in the second row can just talk as they wish, straight ahead. The people in the first row we'd like to turn around and speak towards the audience and that way they can record. Now, in the question and comment period, if anybody has a question or comment, this microphone on this side, my left-hand side, can be brought to the end of the table and he can put his question or comment into that microphone.

Another thing, we have a pointer here. All you do is lift it up like this and there is a flashlight in it and in this way if it becomes too dark, if you have white on black slides or something like that, you can see against the board very plainly.

Now, the first speaker had his Masters from the University of Melbourne, Australia in 1938. He obtained a Ph. D. from the University of London, England in 1940 and a Doctor of Science in 1949 from the University of Glasgow, Scotland. I have followed his work for quite a few years in the days when I was a graduate student. I have always been interested in his work and I think he can attest to that. His fields of interest have been organic syntheses, electronic spectrophotometry, some phases of cancer chemistry. In fact, I think he started out in a synthesis of something like anthracene benzanthracene compounds as possible anti-carcinogens. I think he's had a very illustrious career and he has had some very fine research out. He has a book that has either come out or is coming out soon called "The Chemistry of Heterocyclic Compounds." Dr. Badger.

DR. BADGER: Well, thank you very much. And may I say first of all a word in reply to Dr. Sawicki's and Dr. Stern's words of welcome. I am sure I am speaking on behalf of all the participants in Cincinnati in saying that they are very pleased indeed to be here and we are sure it will be a very fine conference.

When I was a young man in 1938, as Dr. Sawicki has mentioned, I went to London, England to do my Ph. D. with Dr. Cook and Kennaway and I was working on the synthesis of polycyclic aromatic hydrocarbons at this time. Well, in 1938, this was only five years after the isolation and the synthesis of benzopyrene and I cannot remember at that time any suggestion by the workers in that institute that benzopyrene might be connected with any other form of cancer than occupational cancer is. It had, of course, been isolated from coal tar and there was an implied suggestion that it might be present in soot, although it hadn't been very definitely proved at that time, but there was suggestion that the bensopyrene was connected with the so-called spontaneous cancers in the population at large.

However, since then the situation has changed completely. As we all know, bensopyrene has now been identified in a very great variety of materials and there is now a belief that it may be intimately concerned with quite a number of different types of cancer in the population so it seemed to us that we ought to try and find out how this benzopyrene is formed in human environment and perhaps not only bensopyrene but also some other polycyclic carbons, some of which are also cancer producing.

So we decided that we should try and find out if we could how this bensopyrene is formed. When we looked in the light we weren't able to get very many clues. Kennaway in the early days of carcinogenic hydrocarbons had shown that there is a difference in the carcinogenic activity of certain coal tars, some of which had been obtained from coal at a low temperature he found to be either inactive or only very slightly active, and other tars which had been produced at a higher temperature he found these to be quite cancer producing. So that it did look as if the temperature was important.

Then again we have the situation with shale oil. It's been shown that if shale oil is extracted from the solid shale with solvents it is not carcinogenic and there is no bensopyrene in the shale. If, however, the oil is obtained by heating, by pyrolysis of the solid shale, then it becomes carcinogenic and bensopyrene can be detected. So here again the temperature seems to be important.

In petroleum, some petroleums it seems are cancer producing but quite a number of them in the early days have been shown to become cancer producing after being heated. Many of the crude petroleums or petroleum fractions were not carcinogenic when tested in the crude state or in a refined state, but after being heated to a relatively high temperature these became cancer producing.

Kennaway had also found that he could produce certain carcinogenic tars by pyrolysis. He had pyrolysed things like skin and yeast and cholesterol and he had also repeated the experiment which Berthelot had carried out in the 1860's. The French chemist Berthelot had pyrolysed acetylene and, of course, this is one of the classical experiments in organic chemistry because he obtained benzene and a number of other aromatic hydrocarbons and, of course, his work in 1860 was really quite remarkable in that he separated with the primitive equipment then available several hydrocarbons, which one can only admire in retrospect.

One can imagine, for example, carrying out fractional distillations in retorts with the thermometer inserted in the liquid.

Kennaway had repeated this pyrolysis of acetylene in a heated tank of tar which certainly contained benzene and some other things and found that this was cancer producing and here again he was able to show that the temperature was important and he was able to indicate that the carcinogenic material is a hydrocarbon because only acetylene had been used. There was an implied suggestion here that benzopyrene was present in this tar but it wasn't proved at this time. And we had another look at this tar ourselves a couple of years ago when we pyrolysed acetylene andcarried out complete separation of the tar and were able to show that it did contain two percent of benzopyrene and we fully identified it in this case.

Acetylene, of course, may very well be important because as we know it can sometimes be found as a product of partial combustion of quite a number of organic materials.

Now, finally in a preliminary survey we knew about the tar which Kennaway had also used called "Schroeter tar". Schroeter had produced a synthetic tar by treating tetralin with aluminum chloride and he had obtained this crude tar which he showed to be cancer producing and which had the fluorescence spectrum characteristic of benzopyrene. Here again it wasn't known that benzopyrene was present except for this indication from a rather diffuse fluorescence spectrum and we have again repeated this and shown that this tar also definitely contains benzopyrene in quite large amounts. In fact, it can almost be used as a method for the preparation of benzopyrene you can get so much out of it.

May I have the first slide now, please? This is the possible mechanism for the formation of benzopyrene from tetralin. Under the action of the aluminum chloride the bond may break here and link up with another molecule of tetralin to give the compound on the top right of the slide. This compound has been definitely isolated from this mixture. It was isolated initially by Schroeter and it's been confirmed since.

Now the attack of this fragment on the second molecule of tetralin here is in the beta position of tetralin but it is reasonable to suppose that a certain amount may combine in an alpha position and if this is so then it is easy to suppose that under the action of the aluminum chloride that we may remove a few hydrogen atoms, produce cyclisation and hence benzopyrene.

Now this was, therefore, the starting point in our hypothesis for the mechanism which we proposed, working hypothesis for this scheme which we started. We proposed as a working hypothesis and it wasn't necessarily the only way in which benzopyrene could be formed, we supposed that a two-carbon unit such as acetylene might be converted under the influence of heat into a four-carbon unit and that this would be thattwo such four-carbon units could combine to form what we might call $C_6 - C_2$ unit, which is just off the right-hand corner. And addition of another two-carbon unit would give a $C_6 - C_4$ unit. This is the C_6 and the C_4 unit, and I haven't put in the hydrogen atoms because this would not appear at first sight to be very important.

Now, such a $C_6 - C_4$ unit could readily cyclised produce say tetralin or naphthalene, let's suppose tetralin, and the interaction of a $C_6 - C_4$ unit with a tetralin unit would give an intermediate similar to the one which we mentioned in the case of the "Schroeter tar" and that this would cyclise to benzopyrene under the influence of heat.

Now, this was then the working hypothesis which we used. It does not necessarily represent the only path by which benzopyrene can be formed but it seemed to us that it might represent a path and it presupposes that we should be able to identify or find benzopyrene in any tars produced by the pyrolysis of any of these supposed intermediates. That is, that if we started from acetylene we should be able to produce some benzopyrene amongst the mixture that we got. If we started from, say, butadiene, a C_4 unit, a typical C_4 unit, or if we started from, say, styrene or ethylbenzene, which is a typical $C_6 - C_2$ unit, pyrolysis of all these things would be expected to yield benzopyrene. But it doesn't necessarily suppose that everything must be broken down to acetylene before it is resynthesised to benzopyrene.

We can imagine, for example, that a long chain saturated hydrocarbon of paraffin might be broken down to a C_{10} unit. It would be broken down to quite a number of different fragments, of course, but the C_{10} units might cyclise to give us $C_6 - C_4$ units and that in other words synthesis of benzopyrene would in effect start from half way in the scheme which we have here.

So we set out, therefore, to pyrolyse a number of typical compounds in this working hypothesis. A C_2 unit, acetylene, representative of a C_4 unit, butadiene, a $C_6 - C_2$ unit, vinylcyclohexene, and vinylcyclohexene is obtained by the dimerisation of butadiene. One can form vinycyclohexene very readily from butadiene.

Another typical $C_6 - C_2$ unit would be ethylbenzene and another one styrene. All of these were pyrolysed. A $C_6 - C_4$ unit, butylbenzene, was pyrolysed as was also tetralin, another $C_6 - C_4$ unit. And the double unit, the $C_6 - C_4$, $C_6 - C_4$ unit, phenylbutylnaphthalene. So we then carried out the pyrolysis of all these materials by passing them through a tube of silica at 700°, isolated the tars, and then separated and analyzed the tars to see whether we could identify benzopyrene and other aromatic hydrocarbons. In many cases it has been possible to identify as many as fifty different compounds in these hydrocarbon tars and to work out their approximate concentrations.

In trying to understand what happens in thesepyrolitic reactions we must try and understand the first reaction that occurs. And here we are to some extent helped by our physical chemist colleagues because they have carried out quite a lot of work on what may be termed bond dissociation energies, the energies required to break given bonds. And as a very rough approximation we may say that carbon hydrogen bonds have got a bond dissociation energy of about a hundred kilocalories, and that carbon carbon single bonds have got a bond dissociation energy of about eighty kilocalories, and that double bonds are very much stronger, very difficult to break.

Therefore, we can get a fairly reasonable summary of what bonds we may expect to break in any hydrocarbon to give us free radicals and then we may try and work out from these proposed primary free radicals what hydrocarbons we would expect to get.

I am now going to go through quite a number of the different reactions which have been observed, and I will start with benzene because here we have only the benzene ring and the only bonds which can possibly break here are the carbon hydrogen bonds and to some extent the aromatic carbon carbon bonds. Now, this pyrolysis of benzene has been done several times before but we have repeated this with the best techniques we have and we have now been able to show, and prove conclusively, that benzene is a remarkably stable compound but that to some extent bond dissociation does take place, that carbon hydrogen bonds do break, and that if you break a carbon hydrogen bond in benzene you will get phenyl radical. And what would you expect the phenyl radical to do but combine with another molecule of benzene to give you biphenyl, and this is found to be the case. In the tar which we obtained 20.84 percent of this tar consists of biphenyl and most of the remainder is unchanged benzene. The major product is biphenyl.

Now, this I think can only be formed from phenyl radicals on benzene with removal of yet another hydrogen during the reaction. But you might expect that this sort of reaction would occur to a greater extent as well, that you would get further phenylation of biphenyl to give terphenyl, and the paraterphenyl is also obtained in 1 percent yield, metaterphenyl, that is with the second phenyl radical attacking the meta position, this is formed in very small amount, 0.37 percent yield. We could not get very definite evidence for the presence of the orthoterphenyl but we think this is probably due to the fact that this was in a situation where cyclisation can occur very readily to give us triphenylene, the compound in the bottom right-hand corner of the slide. And triphenylene was obtained nearly 0.7 percent yield.

Now, there were a few other hydrocarbons also found to be present in this tar but altogether they totaled only 0.5 percent, so the major product here is biphenyl and to a small extent the products of further phenylation.

Now, similar reactions have been carried out by Lang and Buffleb in Germany on the pyrolysis of naphthalene and the major products here are the binaphthyls. We can get the different positions of substitution here. We get a naphthyl radical and this could attack either the alpha or the beta position. So that we get both alpha alpha, beta beta and alpha beta binaphthyls. But here we can get further cyclisation because the situation is such that further hydrogens can be removed without too much difficulty. If we remove two further hydrogens from the alpha positions of alpha alpha binaphthyl we get the compound in the top of the slide which is perylene, and this is another product of the pyrolysis of naphthalene. If the hydrogens are removed from other positions we can get these other hydrocarbons which we have on the bottom part of the slide which are benzofluoranthene, 10,11-benzofluoranthene and 11,12-benzofluoranthene, which were also isolated from the naphthalene tars.

We have also found all these compounds, the binaphthyls, the perylenes and the benzofluoranthenes from all the tars which have been produced by the pyrolysis of things which give naphthalene in large quantity. Now there is the pyrolysis of tetralin, gives all these compounds as you might expect by just removal of the hydrogen atoms to give the naphthyl radicals and the reaction as mentioned.

These compounds are also obtained from the alkylbenzenes which also produce naphthalene in relatively large amount, from butadiene and from vinylcyclohexene, and it seems to us that this is the way they are formed, that is the naphthalene is first formed and the naphthalene loses the hydrogen to give a naphthyl radical and that this attacks another molecule and so on.

Now, what would happen

if we had a phenyl radical attacking another hydrocarbon, not just benzene but another hydrocarbon. If, for example, we have a mixture where we have naphthalene and phenyl radicals then we might expect the phenyl radicals to attack the alpha position, perhaps predominately, to give alpha phenylnaphthalene and that this would be in a situation where further cyclisation could occur readily to get fluoranthene on the top right of the slide.

If fluoranthene is further phenylated in that position and the cyclisation occurs again in exactly the same sort of way, then one might expect to get a hydrocarbon on the bottom of the slide and here again these compounds, fluoranthene and this compound on the bottom of the slide, ortho phenylenefluoranthene, these compounds have been obtained in all the tars where we have a reasonable amount of naphthalene and also phenyl radicals.

Now, we can come to the phenylation of phenanthrene and here we are assisted by some work carried out by two of my colleagues, Dr. Beckwith and Dr. Thompson who carried out some ordinary phenylation reactions on phenanthrene producing the phenyl radicals in the normal sort of way, that is from benzoylperoxide or from diazoaminobenzene and they carried out the phenylation at about a hundred degrees, isolated the different phenylphenanthrenes and determined their relative amounts. They found that they got no phenylation at the four position of the phenanthrene but that small amounts of phenylation occurred at the 2 and 3 positions but that relatively the amount of phenylation occuring at the one position was quite large. These are relative figures here, and the major part of the phenylation occurred at the 9 position, relative 6.5 to the others. So that phenylation predominately occurs at these two positions.

Now, if we then try and work out what we get if we phenylate phenanthrene, if the phenylation occurred at the 9 position which has been shown by Beckwith and Thompson to be predominately attacked under 100° conditions then we get the 9-phenylphenanthrene and the situation is similar to the one we had before with fluoranthene where cyclisation could be expected to occur very readily to give the carcinogenic hydrocarbon 3,4 benzofluoranthene.

One can also imagine that radical attack from other radicals could occur on phenanthrene or indeed on naphthalene or benzene. If we have a situation where we have butadienyl radical this could perhaps occur at the 9 position also and eventually cyclise to give us triphenylene.

Now, we can get the same

sort of understanding of the method for the formation of these hydrocarbons in pyrene. Pyrene is normally attacked with most reagents in this position where I have a phenyl group now so that the phenylation of pyrene might reasonably be expected to give this phenylpyrene and here again our situation is such that sterically the arrangement is suitable for further cyclisation and this ortho phenylenepyrene might reasonably be expected from pyrolitic reactions where we have pyrene and benzene or pyrene and phenyl radicals and this has been found to be the case.

We have isolated this latter hydrocarbon in several cases and Lang and Buffleb have also isolated this hydrocarbon from a mixed pyrolysis of benzene and pyrene so that there is reasonable expectation that it is formed in this way.

Now, so far we have been talking only of the fission of carbon hydrogen bonds and, of course, our carbon carbon bonds here are all aromatic bonds and, therefore, very stable so you do not normally get very much breakdown of the benzenoid rings, and benzene itself, as I mentioned, you only get a 0.5 percent yield of all the other hydrocarbons altogether. But you can detect the two-carbon fragments, that is you can detect ethylene in the exit gases but not to any very great extent. So the aromatic ring is very stable except that the carbon hydrogen bonds can break and you can get your cyclodehydrogenations without too much difficulty at 700°.

At lower temperatures, I might add this sort of fission of carbon hydrogen bonds occurs very much less readily and you can get only a very small yield of biphenyl if you carry out a pyrolysis at, say, 500°. At 700° you get as I mentioned before under air conditions 20.84 percent yield of biphenyl, but at 500° or thereabouts you get only a very small amount indeed.

Now, let us come to the type of hydrocarbon where we have a side chain and this is one of the first, indeed the first, substitute of benzene which we pyrolysed at 700°, styrene because it looked as if it might an interesting intermediate in our proposed hypothesis.

Now, here we have a benzenoid ring which we would not expect to break and we have a double bond which we would also not expect to break because the bond dissociation energies of the double bonds are very large and therefore can only be expected to occur to a very small extent even at very high temperatures. The bonds which we would expect to break are the bonds between the aromatic ring and the side chain and the carbon hydrogen bonds. If we break a carbon hydrogen bond then we might get radicals as I have on the left-hand side. Loss of a hydrogen atom from the alpha carbon would give this radical. The loss of the hydrogen from the beta carbon would give the next one and we might also lose the hydrogen from the aromatic ring. If the bond between the aromatic ring and the side chain breaks then we will get phenyl radicals and vinyl radicals.

Now, when we pyrolysed styrene, therefore, these are the expected primary radicals and we can see whether we can work out the expected products that we might get in large amount. If we have a vinyl radical as I showed on the previous slide that this might reasonably be expected, a vinyl radical, and, of course, we have plenty of styrene in our mixture, that the vinyl radical might reasonably be expected to combine with the excess styrene to form naphthalene and naphthalene was obtained in over six percent yield in this tar.

If we have loss of a hydrogen atom from the alpha carbon atom we have this styryl radical and this might be expected to combine with excess styrene to give the product on the right of this slide and eventually 2-phenylnaphthalene. And here again 2-phenylnaphthalene was one of the three or four major products of this reaction.

If we have phenyl radicals and the phenyl radicals may be expected if you just lose the side chain from the sytrene and this would be expected to combine as shown in this slide to give stilbene and if it takes up a couple of hydrogen atoms, bibenzyl, or it might cyclise to give phenanthrene; and stilbene, bibenzyl and phenanthrene were obtained in quite large amounts from this pyrolysis. Stilbene in about one and a half percent yield, bibenzyl in about one percent yield, and phenanthrene in quite large amount, over eleven percent yield.

There are one or two alternative reactions which we might expect, but I think these are the major ones. These are the major radicals that you would expect from the pyrolysis of styrene, the initial primary radicals and if they attack the excess styrene that you have in your reaction mixture then you can reasonably explain the three or four major products that you identify in this pyrolitic tar.

Just as an example of this sort of alternative reaction which may occur to a small extent, we might imagine that some benzene has already been formed from the phenyl radical plus hydrogen and that this is attacked by a styryl radical and we would get the same sort of product. But, of course, there will always be more unchanged styrene in the mixture or one might imagine that there might be always more styrene in the mixture so that the former reaction to my mind is much more likely to occur to a greater extent. One can also explain without too much difficulty the formation of a number of other products which I haven't mentioned here, namely: benzene, phenyl radicals formed from the breaking of the side chain from the styrene, phenyl radicals simply have to take up a hydrogen from almost anywhere and, of course, there will be quite a lot of hydrogen in the mixture, to give you benzene, and this was quite a notable constituent of the tar. If the styrene radicals take up excess hydrogen we can expect to get ethylbenzene and this was also found. And one might also expect since you have phenyl radicals to get biphenyl and this was also a product from the pyrolysis.

The formation of pyrene is perhaps a little difficult to explain and this is one possible way in which it might be formed. That is by interaction of styrene with a styryl radical where the hydrogen has been removed from the benzene ring to give you this proposed intermediate which would then undergo further cyclisation. I must admit that we are still a little uncertain as we'll see later in the lecture just how the pyrene is formed.

Now, we did obtain from this reaction a certain amount of toluene, over three percent, about three and a half percent of toluene from the tar, from the pyrolysis of ethylbenzene. And at first sight this looked as if, contrary to our expectation, the double bond had broken, the double bond in the styrene had broken to give you the hydrocarbon but only one carbon attached the benzene ring. However, on further thought it looked as if this might be formed in a slightly different way and this is the mechanism which we believed to be operating in the formation of toluene from pyrolysis of styrene, namely: that we get as I mentioned earlier some bibenzyl or stilbene, both of these things, and the bond dissociation energy of the middle bond in bibenzyl, this carbon carbon bond in bibenzyl, is very low indeed. It's unusually low. It's very easily broken and this might be expected, therefore, to give us benzyl radicals which could take up hyrdogen to give us the toluene found.

In any case, the only methylated hydrocarbon found in any considerable amount was the toluene. We get only very small traces of say, methylnaphthalenes.

When we come to a slightly more complicated hydrocarbon, we chose phenylbutadiene. Here we have a $C_6 - C_4$ unit as we mentioned in our original hypothesis and we can try and predict which way this compound would break, which bonds here would break to give us radicals. Here again the benzene ring would not be likely to break to any extent nor, we think, would the carbon carbon double bonds.

However, we have two carbon carbon single bonds which may differ slightly in their bond order and we calculated the bond orders here. This is the double bond character of these single bonds because being conjugated they have some double bond character and the bonds with the lower double bond character would be predicted to be the bonds which might break easier. Consequently, we would expect this bond to break more readily than the other one.

However, in addition to this fission of the carbon carbon single bond we would expect fission of carbon hydrogen bonds. Now, if we lose a hydrogen from the omega carbon we get this phenylbutadienyl radical. If we just split off our side chain, that is break this bond which I mentioned would be most readily broken, we get phenyl radicals plus butadienyl radicals and if the center bond breaks we would get a styryl radical plus a vinyl radical, and we can see whether these radicals will explain the hydrocarbons which we indeed find in this tar.

The C_6 - C_4 arrangement of atoms in this molecule, of course, makes it very easy for naphthalene to form. And naphthalene was far and away the major product in this pyrolysis so that we have to consider not what would occur by attack of radicals on the phenylbutadiene but what would happen by attack of these different radicals on naphthalene, which as I say is the major constituent. Now, if our naphthalene is attacked by phenyl radicals, as we mentioned a little earlier, we would expect to get phenylnaphthalene and on cyclisation, cyclodehydrogenation at the high temperature involved, the fluoranthene, and fluoranthene was obtained in that tar.

If we attack naphthalene by a butadienyl radical and this is, of course, present if the whole side chain falls off, we would expect to get some phenanthrene formed in this way. And if our phenylbutadienyl radical attacks a molecule of naphthalene we would expect to get this intermediate and on loss of a couple of hydrogen atoms by cyclodehydrogenation we would get the benzopyrene; and on the pyrolysis of this phenylbutadiene we did indeed obtain some phenanthrene, some fluoranthene and benzopyrene and other hydrocarbons also, of course.

The phenanthrene was obtained indeed in a 13 percent yield; and fluoranthene, nearly 4 percent yield; benzopyrene only in small amount but, of course, one would expect the intermediates to be rather unstable and to undergo further bond fission. Phenylphenanthrenes were also obtained, and as you might expect from the earlier slide, 3,4-benzofluoranthene and hydrocarbon benzofluoranthene.

Now, we have also carried

out the pyrolysis of a number of other hydrocarbons, especially the ones I am mentioning now which include both carbon carbon saturated bond and carbon carbon double bond, that is the pyrolysis of ethylene and vinylcyclohexene. Indene was at one time used a s a preparative method for chrysene. We were interested to see whether it would give benzopyrene under pyrolitic conditions of 700°. Which bond would we expect to break here?

Firstly, the benzenoid ring being very stable we would not expect to break to any extent, nor would we expect any of the double bonds to break. But the carbon carbon bond would be very weak, the bond dissociation energy being lower than the bond dissociation energy for carbon hydrogen, so that the bonds which you would expect to break are these. And, therefore, you can work out which radicals you might expect from this pyrolysis and see whether you can combine these radicals to get the products which you observed.

Similarly, in tetralin, all those bonds on the saturated ring might be expected to break to some extent and in the vinylcyclohexene in the same way.

This is a slide representing just the alkylbenzenes which we have studied, that is, toluene, ethylbenzene, propylbenzene and butylbenzene. Now, in toluene there are any two bonds which we might expect to break to any extent, the carbon hydrogen bonds, especially in the side chain, and the bond between the phenyl ring and the side chain. This has relatively low bond dissociation energy and because of the stability of benzyl radicals the bond dissociation energy for the carbon hydrogen bonds in the side chain of toluene is also very low. So that benzyl radicals and phenyl radicals would be expected to occur or to be produced very readily in this pyrolysis.

Now, similarly in the pyrolysis of ethylbenzene we would expect to break carbon hydrogen bonds in the side chain especially, break the center bond here, carbon carbon bond, and break the bond between the phenyl ring and the side chain. And the same sort of thing might be expected for the other ones. I mentioned butylbenzene particularly because here we have quite a number of opportunities and the saturated carbon carbon bonds it seems are not broken equally easily because the pyrolysis of butylbenzene gives rather a large amount of ethylbenzene and rather a large amount of benzene. There seems to be a preference to break off in two-carbon units.

Similarly in propylbenzene you get a very large amount of toluene. That is, there seems to be a preference for a two-carbon unit to come off rather than a single carbon unit. So that in all these cases we would expect to get things like phenyl radicals, benzyl radicals, phenylethyl radicals and so on.

Now, on the pyrolysis of all these compounds, the indene, the tetralin, vinylcyclohexene, all the alkylbenzenes, all of these compounds gave benzene, so that the side chains are easily removed, and all of them gave some naphthalene. So chain re-synthesis as we may term it must occur. We must be able to build up a side chain, because if you get naphthalene by the pyrolysis of toluene it must add some carbon atoms to the side chain somehow or other.

And this is a slide indicating possible ways in which this can occur. That is, if you have a phenyl radical and this attacks ethylene which may be present in your reaction mixture, then you can get your phenylethyl radical. If you have a benzyl radical in the next line and attack ethylene you get your phenylpropyl radical and so on. It seems that the side chain can be built up without too much difficulty.

Now, finally in this preliminary section I would like to mention that we pyrolysed a $C_6 - C_4$, $C_6 - C_4$ compound, that is a $C_6 - C_4$ Phenylbutylnaphthalene, and naphthalene being also a $C_6 - C_4$ unit. Now, these structures I have written on this slide are all exactly the same except that I have bent the bonds in different ways. If it is bent in this way it's easy to see that you might expect to get some benzopyrene by removing hydrogen atoms from those positions. And on the pyrolysis of this compound you do get a small amount of benzopyrene.

Now, written in the form on the top right of this slide you might expect to remove hydrogen atoms to get some perylene and perylene is also obtained by the pyrolysis of this compound. And as written in the bottom of this slide, we get phenylphenanthrene, and indeed under certain conditions one can pyrolyse this compound and get a very good yield of phenylphenanthrene indeed.

I mention this simply to illustrate the fact that we can be reasonably sure, I think, that the sort of reactions which we are discussing do, in fact, occur under pyrolitic conditions.

Now, the various pyrolyses that we have carried out enable us to come to a reasonable conclusion about some alternative mechanisms. When we first started this work we felt that it might be formed by the hypothetical mechanism which we have suggested, or that the benzopyrene might be formed in some other ways.

For example, if we formed chrysene in some fashion or other and then add a C_2 unit to this, that is, if we attack crysene with a vinyl radical we might expect it to go into this position as shown and then further cyclisation would give us our benzopyrene. Well, in fact, I think we have been able to exclude this mechanism as a major source of benzopyrene because of the variation in the yield of the different compounds.

The pyrolysis of indene, for example, gives a very large amount of chrysene and ethylene is found in the exit gases but it gives practically no benzopyrene. And so our discussion of the relative amounts of the hydrocarbons formed in any pyrolitic tar enables us to exclude this, I think.

Another possible way in which the benzopyrene might be formed is from the intermediate benzanthracene which we may suppose to be formed by an unknown mechanism by addition of a C_2 unit at the top of the molecule.

Another one would be pyrene plus a C_4 unit to form the ring on the side there. Now here again we think that we can exclude these as major mechanisms for the formation of benzopyrene by discussion of the relative amounts formed in different pyrolyses.

We recently started, however, to see whether we could get some rather better evidence on these mechanisms by reaction or the pyrolysis of labeled hydrocarbons. When we started this with labeled ethylbenzene, a radioactive carbon in the alpha carbon atom, which is shown with an asterisk, now when we try and predict what radicals we would get when this labeled ethylbenzene is submitted to pyrolysis this slide is a summary of what we would expect.

On the right-hand side of the slide, if you imagine when the whole side chain falls off then you would get a phenyl radical plus an ethyl radical with one labeled. The phenyl, of course, would be unlabeled. The ethyl radical might take up an extra hydrogen to give labeled ethane and this might break to give you both labeled and unlabeled methyl radicals.

The alternative way in which the bonds can break is that the carbon carbon saturated bond might break as shown in the middle of theslide to give a labeled benzyl radical and an unlabeled methyl radical. The labeled benzyl radical would give you a labeled toluene and this could also be expected to split to some extent under high temperature conditions to give you unlabeled phenyl and labeled methyl.

Now, again we might remove the hydrogen atom, that is break a carbon hydrogen bond to give us a labeled phenylethyl radical and another hydrogen atom give us labeled styrene and this might break also to give us unlabeled phenyl and labeled vinyl radicals.

The major things then I would like you to note are that the phenyl radicals are always unlabeled, the two-carbon units must always be labeled but the single-carbon units, that is the methyl radicals, can be unlabeled, labeled or as shown on the right-hand side of the slide, labeled or unlabeled.

Now, we can see whether the results which we get on radiochemical analysis of the products agrees with our prediction therefor. The isolated benzene from this pyrolysis formed in 34.8 percent yield and the isolated biphenyl formed in just over three percent yield, these were found to be substantially inactive and agree with formulation that they are prepared or their mechanism of formation is that the phenyl radicals simply combine with hydrogen to give you a benzene or the two phenyl radicals combine to give you a biphenyl. When I say these are inactive, the analysis ineffect showed approximately 0.04 of an atom, 0.04 of a labeled atom in the case of benzene and approximately double this in the case of biphenyl.

To all intents and purposes, however they are unlabeled.

Now, thetoluene, our mechanism for the formation of toluene we can have several choices. We might suppose, for example, that benzyl radicals which would have to be labeled according to our predicted fission, that the benzyl radicals take up a hydrogen to give a labeled toluene.

Another way is that a phenyl radical could combine with a labeled methyl radical to give you labeled toluene or a phenyl radical could combine with an unlabeled methyl radical to give you unlabeled toluene. And, in fact, the experiment showed that we had 0.74 labeled atoms in our toluene so we conclude that all of these mechanisms are operating to some extent.

Now, the styrene, we can imagine that the styrene is formed from the ethylbenzene by loss of two hydrogen atoms by an intermediate radical and since our initial compound has one label the styrene would also be expected to have one label. In fact, we found experimentally 1.08 labeled atoms.

Here we have the explanation, we think, of the chain re-synthesis, the way in which the carbon atoms can combine with one another to form extra rings. We have the formation of naphthalene, for example, from ethylbenzene and this could occur in several ways similar to the one which I have here.

That is, we can suppose that a phenyl ethyl radical which would have one label might combine with some ethylene produced from another molecule and this ethylene would also have one label. So that our intermediate radical would have two labels and on cyclisation and naphthalene would be expected to have two labels, experimentally 1.99 labeled atoms were found.

Now, in the case of phenanthrene we have a couple of possible alternative mechanisms. If we suppose that ethylbenzene is broken down into benzyl radicals and that two benzyl radicals combine, then both of the benzyl radicals should have a label so that bibenzyl on the right of the slide should have two labels and the phenanthrene formed by cyclisation from those should also have two labels .

There is an alternative mechanism, however, by which the phenanthrene could be formed from a $C_6 - C_2$ unit such as styrene which would have one label and a phenyl radical from another molecule of ethylbenzene. If this reaction occurs then we might expect to get a stilbene or a bibenzyl as an intermediate which would have only one label and therefore the phenanthrene should only have one label.

So we have these two alternative mechanisms, therefore, one of which would give a phenanthrene with two labels and the other with only one label. In fact, the phenanthrene isolated had only one label and, therefore, we presume that this mechanism is the one which is occurring predominately in this pyrolysis.

We come now to the method for the mechanism for the formation of 3,4-benzofluoranthene. Phenanthrene has only one label and according to the mechanism which we suggested for this hydrocarbon we might have attack by a phenyl radical on our phenanthrene and would attack predominately in the 9 position, to some extent also in the 4 position, according to those figures as shown earlier. But whether it attacks in the 1 position or the 9 position we would eventually get this 3,4-benzofluoranthene.

The one on the right-hand side is the compound which you would get if it attacks in the 9 position. Now, therefore, the predicted radiochemical labeling for this 3,4-benzofluoranthese is one labeled atom, and that is found to have only one labeled. It seems, therefore, that this may very well be the mechanism for the formation of the 3,4-benzofluoranthene.

I may mention incidentally that this particular pyrolysis gets quite a large amount of phenanthrene, 14.3 percent, and there is a reasonable amount of 3,4-benzofluoranthene also formed and the experimental value was 0.91 labeled atoms which was fairly reasonable for this sort of way.

When we come to the possible mechanism for the formation of chyrsene we have several alternatives and the one which I would like to draw your attention to most is this one on the bottom here. We can suppose that we have an interaction between a $C_6 - C_2$ unit such as an ethylbenzene molecule itself or a radical derived from it which would have one label and a $C_6 - C_4$ unit which could be also the intermediate for naphthalene which would have the original label and the label from the second molecule or the second two-carbon unit. And these would be expected to combine in this sort of fashion by cyclisation here to give us a chrysene with three labels.

But as I indicate briefly on the top of this slide there are other alternative ways in which this hydrocarbon could be formed from not too dissimilar units which would also give chrysene containing three labels.

Indeed, very experimentally it was found that chrysene contained 3.1? labels and we are not able to separate these mechanisms but it looks as if something rather similar to this one is occurring.

Let me just conclude by showing the sort of apparatus which we have used for the great majority of our pyrolitic work. This is the diagrammatic representation of our furnace which has a silica tube running through another silica tube which bears the electric wiring for the heating and the temperature measurement. The compound is added to the tube by this device here and there is a carrier gas, nitrogen, which is purified in these devices.

And on the right-hand side of the slide we have a collection apparatus for collecting the tar as it is formed. The tube is on a slide incline ten degrees to facilitate the run-off of the tar.

Now, in concluding my talk I would like to make acknowledgment to my collaborators in this work, all of whom have contributed significantly to this work which I have described over the last six years, and also to the Damon Runyon Foundation which had provided most of the financial support.

Thank you very much.

CHAIRMAN SAWICKI: Are there any questions or comments on Dr. Badger's paper?

DR. FALK: Dr. Falk, UC. Dr. Badger, of all the chemicals that you ever worked on, have you ever come across a few others that you just didn't mention because of the time shortness, but can you tell us something about 1,2-benzopyrene formation from precursors, because it is always there, and I presume you must have had lots of it too, and possibly some coronene, just to mention two?

DR. BADGER: I am sorry but I can't say terribly much about these because there are quite a number of hydrocarbons which we have not yet been able to devise mechanisms and 1,2-benzopyrene is one of these. We are not too sure indeed how pyrene itself is formed. I really forget to mention the pyrene formation in this synthesis. Pyrene itself has two and a half labeled atoms and it seems, therefore, that it is formed by two different mechanisms, and we haven't been able to isolate enough 1,2-benzopyrene or 3,4-benzopyrene from this one to be able to see how that is made.

Coronene is only formed in very small amounts in all the pyrolyses that we have described and I don't know how it's made.

I should also mention perhaps that we carried out in very recent months the pyrolysis of labeled tetralin and labeled butylbenzene, butylbenzene being a typical $C_6 - C_4$ unit, the label on the amiga (phonetic) carbon atom and the benzopyrene has been isolated in this case and found to have two labels so that this also supports it.

But as far as 1,2-benzopyrene is concerned, the answer is I don't know.

CHAIRMAN SAWICKI: Any others?

DR. VAN DUUREN: Dr. Van Duuren, New York University. Dr. Badger, I would like to ask you whether you have looked into the pyrolysis of aliphatic and alicyclic compounds, for example, steroids?

DR. BADGER: Now, we haven't yet done this.

A few people have, of course, pyrolysed some long chain fatty compounds, waxy material from tobacco and one or two compounds of this sort and I think decane has been done also some years ago but under exactly the same conditions. The information is, I think, incomplete yet but it does look as if saturated compounds break down by carbon carbon fission and that you can get a $C_6 - C_4$ unit in some fashion to combine to form benzopyrene. The steroids we haven't looked at all.

DR. THOMAS: Thomas, University of California, Berkeley, Dr. Badger, I wonder if you could extrapolate out from pyrolysis to self-sustaining combustion or internal combustion with respect to the proposed mechanism, would they hold other than the pyrolysis?

DR. BADGER: This is perhaps a little difficult to answer off the cuff but my reaction would be that it might if you can get the necessary free radicals and I imagine that one could under these conditions that you would get similar reactions occurring.

CHAIRMAN SAWICKI: Dr. Katz?

DR. KATZ: Katz, Ottawa. I am sure, Dr. Badger, that you have considered the possibility of the polymerization reaction occurring and the formation of polycyclics through auxiliary mechanisms which would, of course, involve the free radical hypothesis and then possibly at lower temperatures than the actual pyrolysis the formation of these condensed compounds through polymerization reaction? Do you think there is anything in this hypothesis?

DR. BADGER: Well, the only information we have indicates that you don't get the polycyclic aromatic hydrocarbons at low temperatures to any signficant extent.

DR. KATZ: But you do get the free radicals and the free radical theory is one of the important theories in polymerization. I was suggesting that initially the free radicals are formed and these are present, as the combustion points cool down more or less radically you could get some of these polycyclic compounds formed through the polymerization of free radicals. For example, the free radical of styrene; for example, the ethyl free radical, the phenyl free radical and free radicals involving alkyl side chains.

DR. BADGER: Do you, in fact, get free phenyl radicals? I just wanted to know whether this was so, whether the only form of radical you get -- I don't know anything about this, you see, I just wondered if the only radical you get is by opening a double bond. Do you ever get a radical with the loss of a hydrogen atom?

DR. KATZ: You do get that in polymerization reaction because one of the ways of explaining polymerization first of all through the famous Stalinger (phonetic) free radical mechanism, is through the formation of a free radical which involves loss of a hydrogen atom.somewhere.

An alternative explanation can be arrived at by postulating a hydrogen wandery (phonetic) mechanism which in effect is not very greatly different from the free radical mechanism.

I recall early experiments that I conducted many years ago with indene, and in the pyrolysis of indene and polyindene. You get the formation of a condensation product involving free molecules.

CHAIRMAN SAWICKI: Thank you, very much.

DR. LINDSEY: Lindsey, Sir John Cass College, London. I should like to ask two questions, very, very brief ones. First, has Dr. Badger produced any quantities of carbon during his pyrolyses and, secondly, has he used temperatures other than the 700 which he has mentioned? I am sure these two things will interest everybody who is present.

DR. BADGER: The amount of carbon varies considerably depending on the hydrocarbon which was pyrolysed. The amount of carbon from acetlyene is very great indeed. In fact, we have a lot of trouble with acetylene and the tube tends to block up and you have to stop the reaction and clean it out again. In some cases it's relatively small. In all our experiments though we have taken the apparatus apart and washed out with solvent carbon. But the answer is that we do find carbon in varying amount.

The second question in connection with temperature, we have used 700° for all the pyrolysis except that on one or two we have used 550° just to see the difference. The main difference which we observed was that the reactions involving carbon hydrogen fission occurred to a very much smaller extent at the lower temperature. The carbon hydrogen fission requires apparently the high temperature.

DR. SAHNI: Sahni, New York University. I would like to know if there has been any theoretical work on the different mechanisms that have been suggested by Dr. Badger, any kind of theoretical work.

DR. BADGER: Well, no. I think that as far as we are concerned we try in a theoretical fashion to predict which bonds will break and we have carried out determinations or calculations of bond orders to facilitate us in this, to help us come to a conclusion. That is, if a carbon carbon bond has a high double bond character we think that it is less likely to break than one with a low double bond character. But apart from this, no.

CHAIRMAN SAWICKI: Okay. Next.

DR. LYONS: Dr. Lyons, recently

scientists at Woods Hole discovered considerable quantities of aromatic hydrocarbon in benzopyrene in one case up to three percent, benzopyrene in the soil of Massachusetts. They considered that the aromatic hydrocarbons in this case were not due to atmospheric fall-out but had a geophysical genesis. Could Dr. Badger suggest any possible mechanism in this regard?

DR. BADGER: No, but I am very interested in this. There is one particularly well-known example with a polycyclic aromatic hydrocarbon occurring naturally with a mercury ore. I have just forgotten the name of the ore for the moment. When you distill over the mercury you can get this hydrocarbon as well so it does occur naturally. Just how it's formed is I think a very interesting scientific problem.

Some of the polycyclic aromatic hydrocarbons in soil could, of course, be produced by degradations from steroids but this is a complete speculation.

CHAIRMAN SAWICKI: Next question? Dr. Thomas?

DR. THOMAS: Thomas, University of California, Berkeley. You made a statement concerning chain synthesis and it seems that side chains can be built up without too much difficulty. We haven't been working in the pyrolysis region, we have been working out in the self-sustaining combustion region trying to find the alkyl derivative of the arene and we have been very unsuccessful. Could you comment on this particular point?

DR. BADGER: Yes, I think that this is true that in air pyrolysis we very seldom get polycyclic compounds having alkyl substituents. We very frequently get toluene, of course, and a little methylnaphthalene but hardly ever do you get significant quantities of the, say, methylated pyrenes. You can sometimes detect very small quantities, we think is due to the fact that the alkyl substituent tends to come off under the pyrolitic conditions; that the bond between the aromatic ring and the saturated carbon is the weakest bond in the compound and that this is just lost as a methyl radical.

Nevertheless, I think that such compounds must have an existence even if it's only for a brief time and it might depend to what extent or how long the hydrocarbon was at the high temperature. There is no doubt, of course, that the chain re-synthesis does occur because we do get naphthalene from things like toluene but you don't get many methylnaphthalenes.

DR. THOMAS: We have worked on the assumption that the bond energies were too high to form these side chains where you have worked the other way taking bond energies for breaking the bond. We were trying to take bond energies on synthesis and we had decided that the energies were just too high to form these side chains.

DR. BADGER: Well, if you pyrolyse a methylated polycyclic compound I think you will find that the methyl group will come off.

CHAIRMAN SAWICKI: Has anybody here isolated any methylated hydrocarbons from any pollution source? Dr. Van Duuren, would you have a comment?

DR. VAN DUUREN: Van Duuren, New York University. We have isolated several methylpyrenes from cigarette tars and I believe that this work has been done before us by Dr. Lindsey and subsequently by Dr. Hoffman. As I recall there were two or three of the methylpyrenes that were present in cigarette tars.

DR. BADGER: About in what quantity, in small quantity?

CHAIRMAN SAWICKI: Any other comment?

DR. HOFFMAN: Dietrich Hoffman, Sloan Kettering Institute, New York City. I have a question, Dr. Badger. In answer to Dr. Lyons' question you mentioned that it might be possible that in the soil from steroids are formed polynuclear aromatic hydrocarbons, but I didn't understand you right. I would like to have your comment, please.

DR. BADGER: I think the answer is that we don't know of any. They hypothesis was advanced in 1935, I think. To my knowledge there has been no evidence presented since that time that this transformation does in fact occur. Nevertheless, I think it's possible that you might get by degradation and sunlight and so on polycyclic aromatic hydrocarbons including steroids in the soil.

CHAIRMAN SAWICKI: Any other questions?

Well, in that case we will take our recess and come back at the appointed time.

(Short recess.)

CHAIRMAN SAWICKI: Will the meeting come to order. If you wish to take off your coats, you may. We don't care.

The next speaker is Mr. Begeman. Mr. Begeman is a senior research engineer with the General Motors Research Laboratories. He was born and raised in Detroit, Michigan, and received a Bachelor of Science Degree in Chemical Engineering from the Detroit Institute of Technology in 1937.

Before the war Mr. Begeman spent nine years with the General Motors Research Laboratories, mostly on the analysis of fuels and synthetic reaction mixtures by fractional distillation.

He was in Chicago for three and a half years where he developed automatic distillation equipment and instrumentation. Since returning to General Motors in 1951 he has been engaged in a variety of projects dealing with the separation, purification and analysis of fuels, oils and exhaust products by physical chemical methods. Mr. Begeman.

MR. BEGEMAN: Thank you, Dr. Sawicki.

This will provide a change of pace from the first paper this morning. I am going to be talking mostly about equipment, but I hope that you will also find this of interest.

General Motors has embarked on a cooperative program with the Sloan-Kettering Institute for the purpose of assessing the contribution of potential carcinogens by automobiles to the atmosphere. On this joint venture the recovery of high molecular weight materials which we will refer to as tar ~~as~~ bar particulate matter from engine effluents and from the atmosphere is being carried out by General Motors. Thus recovered and the biological assay for tumor potentcy of the tar are carried out at the Sloan-Kettering Institute.

This paper comprises a description of the equipment and the procedures which have been utilized at General Motors, the collection of particulate matter from 100° on the exhaust gas and blow-by gas of a modern 364 cubic inch engine and also from the atmosphere.

The paper is divided into two parts. The first part covers the development of the apparatus and the procedure for the collection of the particulate matter from an engine operating in a dynamometer installation and the recovery of tar from this particulate matter.

The second part describes the design and testing of a mobile air-sampling apparatus for the collection of particulatematter from the atmosphere and the recovery of tar from this material.

On these discussions reference is made to tar and it is defined here to be the benzene-soluable organic material which is not volatile at 35° centigrade. So we visualize two techniques that can be used for arriving at an assessment of attributable automobile exhaust gas to atmospheric carcinogens. These we designate as a direct technique and an indirect technique.

In the direct method the rate of emission of carcinogenic material per automobile or per gallon of gasoline consumed is determined experimentally. And by relating this value for a single engine or for a gallon of gasoline burned to the total number of hours of automobile operation or the total quantity of gasoline consumed, the total amount of carcinogens in the atmosphere attributable to automobiles can be estimated.

A modification of this technique permits the assessment of expected concentration of such carcinogenic matter in the atmosphere attributable to automobiles. For example, by relating the automobile emission rates of carcinogen and carbon monoxide of which automobiles may be considered a unique source, and by measuring the atmospheric concentration of carbon monoxide, the expected atmospheric concentration of the carcinogenic material can be estimated.

Then a comparison of this carcinogenic concentration with the atmospheric concentration to direct sampling provides an estimate of a relative contribution of the automobile to other sources and a similar analysis can be made using lead rather than carbon monoxide as a unique source of automobile emission.

Another technique for determining the relative contribution of automobiles is to compare detailed or unique analyses for one or several polynuclear aromatic hydrocarbons in the exhaust gas and those found in atmospheric tar and those finger prints, as they might be called, which would then be developed from these analyses for the particular polynuclear aromatic hydrocarbons. And in this connection it may also be desirable to have similar or comparable finger prints or results from other peculiar sources.

In the indirect method the concentration of carcinogenic material in the atmosphere is measured at a number of locations related to automobile traffic density. The variations in carcinogenic concentration with traffic density permit an indirect estimate of the contribution of the automobile and with this technique measurement of atmospheric concentration of carbon monoxide and lead are again applied, and these provide a criteria of traffic density. And since these components are definitely related to exhaust gas emission they serve as a better index of vehicle emission.

Now, in order to pursue the program by both the direct and the indirect method the equipment for the collection of tar directly from exhaust gas of an engine and from the atmosphere has been constructed and these are items which will be described this morning.

Now, one of the important objectives of the engine experimentation is the determination of the relationships between the quantity of the tar collected and the volume of exhaust gas or blow-by gas and the quantity of fuel used and the length of time the engine is operated, and to our knowledge no one has carried out the recovery of tar in exhaust gas of an engine in a manner which yields these relationships precisely.

The printed version of this

paper will describe briefly the work of several groups, other groups who have recovered polynuclear aromatic hydrocarbon from diesel engines. These efforts have been carried out mainly from the point of view of demonstrating the quality of exhaust gas with respect to these organic materials.

Generally the collection systems which are utilized consisted of one or more traps or a dry filter, but generally not both. And it's been our experience in this work that both a condensed liquid phase and the filtered particulate phase is needed in the recovery for a quantitative approach to the collection of the tars present in exhaust gas from an engine.

Now, these earlier data are very valuable and taken together they conclusively demonstrate the presence of carcinogen in exhaust gas present both in gasoline engines and diesel engines, or they provide little information of the problem as I stated. The principal aim of the work then being carried out in this joint venture with the Sloan-Kettering Institute is the establishment of the quantitative output of carcinogenic materials from automobiles and the determination of the total contribution of such materials in the atmosphere.

Now, our initial exhaust gas sampling for tar particulate from the automobile engine was carried out on a relatively small scale. The objectives of initial small scale operations are two-fold: The development of more suitable methods and materials for the quantitative quantity recovery of particulate matter in exhaust gas and these include evaluation of fuller filter medium for use of exhaust gas, and two, the approximate measurement of benzene-soluble tar in exhaust gas so that the volume of exhaust gas required to be handled could be suitably compromised between equipment size and running time.

In these early tests the engine was operated at the equivalent of 35 miles per hour road load and approximately 7 percent of the exhaust gas was sampled and was taken at a point close to the exhaust manifold on the engine. Some exhaust gas was passed first through a filter and then later through a condensing section, and in other experiments the condensing section was placed ahead of the filter.

In those experiments in which the exhaust gas was taken through the filter media first the temperatures were generally arranged at about 250° Fahrenheit whereas in the experiments in which exhaust gas was condensed first the temperature was generally at room temperature.

The results of these initial experiments can be summarized in five items. The organic material in exhaust gas can best be recovered by first condensing the water phase and some of the tar and other solids in the exhaust gas are also precipitated along with the water phase, which is the main liquid phase condensed. A one-half inch thick all glass filter medium of very fine fibers which can be identified as the Number 50FG filter down made by the American Air Filter Company could be used to retain the particulate matter in exhaust gas and that was finally selected for our use because the initial pressure drop was only about 25 percent over that of the somewhat superior absolute filter method.

It was determined that in using the half inch filter medium we retained a minimum of 96 percent of the particles in the exhaust gas that were capturable on that sort of paper; and three, we recovered about 2 grams of tar; and four, approximately one-third of the total tar was recovered by extraction of the condensed water and it was, therefore, important that this fraction not be neglected.

And finally, in order to recover the tar at the rate required for carrying out the biological program we concluded it was necessary to handle 100 percent of the exhaust gas so the system was built to take care of this.

Now, may I have the first slide, please? The first slide I have summarized briefly the result of our experimentation in which we evaluated certain filter media for use with exhaust gas. Pressure drop in millimeters of mercury versus the filtration time. We know that for our absolute filter the MSA 1106BH paper, the pressure drop was reasonably high to begin with but it declined very, very sharply and the light in our installation would be very short.

The Number 50 FG medium again gave a considerably longer light before the pressure drop rise became prohibitive for our system and the combination of MSA 1106BH and the 50 FG was in between. If, however, we condense a liquid phase before filtration as shown by this curve we extend the effective life of the filter very considerably, something over seven times, seven or ten times the life with MSA filter, or about three times or four times as long as the filter before condensation.

So this is then the arrangement which we built into our large scale system. The fourth curve shown on here is the combination of a somewhat courser medium designated as the Number 25 FG as a pre-filter for the Number 50 FG. We did not use this arrangement because we had decided it would be to our advantage to condense ahead of filtration.

Now, based on the information gained in the small scale operation a combined condensation filtration apparatus was constructed to handle the exhaust gas, total exhaust gas from the 364 cubic inch engine. This is a relatively large V-8 engine used in our larger passenger cars today. This is the only engine which we have used in our experiments to date.

This system as illustrated in the next slide, this is the system which we built to handle all exhaust gas from the engine. The exhaust gas goes first through a diverter valve which functions to direct the gas either to waste as during warm-up or during operation which we sample only during certain phases of a cycle driving program or through the condensing and filtration system.

The cooling system comprises two pre-coolers, one air cooled and one water jacketing which reduced the temperature of exhaust gas coming into the main condenser to about 300° Fahrenheit. Now, that varies according to the driving cycle where the engine is operated at different speeds and loads.

The exhaust gas leaving the condenser is essentially the same temperature as the cooling water which varied in our experiments from about 58 to 70° Fahrenheit. The water and other material that falls out from the gas or condenses from the gas accumulates in this accumulator section which has a capacity of about 20 gallons where it is pumped periodically to a transfer drum of a capacity of about 55 gallons. Then the transfer drum is moved to another location for the extraction operation.

The exhaust gas then which is now slightly about the dew point goes through our filter section and this is the section which contains the number of filters and from there it is discharged.

Now, the photographs of this equipment are illustrated in the next several slides. Here we see the engine, the guard on the front of the engine, the dynamometer for controlling the speed and the load of the engine. This is the pre-cooler one which is air cooled and this is a water jacketed pre-cooler. This is the main condenser. It is about six inches overall diameter and contains about 286 quarter-inch diameter tubes, to provide a large cooling surface. The length is six feet.

The exhaust gas then goes through this accumulator section where the condensed products accumulate. The pump situated right here pumps the condensate to a drum not shown in this picture. The filters are in this area in back of the accumulator section. You can't see the diverter valve in this picture but it's shown in the next slide. A few of the valves which serve the important functions of either directing exhaust gas to waste when it is not wanted or to our collection system.

This is the path from the

engine. Now, the next slide is a view of the filter carriage. It is a 24-inch cube. Actually four are used. There is a capacity for five. We find four is ample. We simply block off the entrance to a fifth section. The filter medium is preheated in an oven before installation in this unit at 700° Fahrenheit for a period of about two hours to destroy the binder which is normally built into the filter medium. Part of the binder is soluable in solvents used to extract the tar so in this we destroy it so as to not later contaminate the tar collected. Now, that's all for that slide. Thank you.

Now, at the conclusion of a run tar is recovered from the exhaust system in three fractions. From the condensed water by extraction with benzene. From the filter medium by extraction with a mixture of benzene methanol ratio four to one. And from the condenser accumulator transfer and drum and other parts of the collection system by rinsing with toluene, by wire brushing the tubes and the condenser and certain other parts of the system.

A piece of equipment which greatly facilitates our handling the large quantities of water and incidentally I might have indicated that the 20-gallon capacity in the accumulator requires about 15 hours of operation on a cycle which I will describe momentarily to accumulate that much water. The column is about two inches in diameter, about six feet long and consists of eleven discreet. Water feed flows through a rotameter for rate measurement, enters the top of the packing, flows downward by gravity against the ascending benzene which flows from this flow meter and rises through the water phase.

They are mixed at eleven discreet places along the way so that the contact is very intimate. The overflow of benzene tar solution then flows out the very top of the apparatus and accumulates in the container at the bottom.

We find that after extraction in that apparatus the water is quite completely free of tar, no further extraction of tar can be effected by further contact with fresh benzene so the filters are extracted in a large steam-heated 17 leader Soxlet apparatus and this is illustrated in the next slide.

Normally we extract the filter for a period of about 28 to 30 hours during which time about 20 to 22 siphoning actions take place. Now, all the solvents that are recovered are extracted in small scale apparatus. And also I mentioned that all the solvents that are used in the operation are pre-distilled before being used. Most of the solvents from the tar solutions are removed from the tar by distillation through the same packed distillation columns and when the tar solution has been reduced to about 500 cc the solution is filtered through a fine glass paper or millepore membrane using a millepore apparatus. And the final removal of solvent then is effected under vacuum at room temperature or slightly above room temperature 30 to 35 degrees using a glass oscillating devise which is illustrated in our next slide, so that we do not have to heat the tar essentially above room temperature.

This is the flask, an air-cooled trap. The rotation is effected by a windshield wiper motor. The trap for condensing the vapors protect the pump, the cloud gauge for reading the pressure. The final evaporation condition is as I said about 35° centigrade and less than one-tenth of a millimeter of mercury maintained for a minimum of three hours.

Now, the volume of blow-by gas, that is the gas which passes the piston rings from the combustion chamber to the crankcase in an engine ranges to about one and a half percent of the exhaust gas volume under cruise and acceleration condition to as high as ten percent of the volume of exhaust gas under part-throttle deceleration.

Now Bennett and others at General Motors have shown that the composition of blow-by gas is about 75 to 80 percent carbureted mixture gasoline and air and about 20 to 25 percent combustion products and inasmuch as the temperature of blow-by is different from that of exhaust gas we decided to recover this fraction also and analyse it for polynuclear hydrocarbons.

The apparatus used to collect blow-by gas is used in the next slide. It's very similar in nature to the collection system for exhaust gas. A vent from the crankcase called the breather tube is connected to what we call the blow-by lines in the engine draft tube through a condenser and accumulator which is submerged in flowing water through filters which are also the Number FG filter with two blankets in series through a flow meter and aspirator. We use an aspirator in this case because of the pressure drop to keep the pressure at the breather tube essentially atmospheric as it is in normal car operation.

In order that representative tar be collected for analysis and for use in biological experimentation the engine is operated on what we consider assimilated city driving schedule and the driving schedule is illustrated in the next slide.

The station or vent shown here consists of eight discreet sections, the driving condition idle, acceleration from 0 to 30 miles per hour, cruise from 30, cruise 50, decelerate to 20, cruise there and back down to an idle condition. The cycle then is automatically repeated over and over again. You can read the percentages of time of each of these particular conditions, the exhaust flow rate and the volume attributable to each of the particular conditions. The values of a hundred cubit feet per cycle in an average rate of 40 cubit feet per minute are we think within 5 percent of what actually is exhausted. The average speed on this cycle is about 23 miles per hour if a car had been going through on the road.

The next slide illustrates the actual accelegraph recording of engine speed and dynamometer speed on our particular driving speed. You notice the engine speed does not equal the car speed because it is driven through a torque convertor. The total cycle is 150 seconds.

The next slide, this is a photograph of the black box, as we call this program, for operating the engine automatically on the driving cycle. You can see in the background the engine, the dynamometer, our tar collection system through the glass window. The fuel consumed was about 11 miles per gallon, equivalent to 11 miles per gallon and the oil consumed was about 1600 miles to a quart and we considered this very typical of a heavy automobile operating under such a driving schedule on the road.

Now, the exhaust gas rates

were measured directly. They were also calculated in fuel rate measurement and exhaust gas analyses. This is the equipment. It is not intended to discuss in detail at this time the results that have been carried out using the equipment that has been described.

It is anticipated that the results as to tar recovery rates from both exhaust gas and blow-by gas as well as a preliminary assessment of the contribution of the automobile to atmospheric carcinogens will be presented at the Society of Automobile Engineering meeting in January of 1962.

The next slide illustrates or summarizes the results of tar collection from exhaust gas system, tar condensed from water phase by extraction of benzene averages about 28 percent of the total. Extraction of exhaust gas using benzene methanol about 46 percent, and pyrenes of the exhaust system with toluene about a quarter of the total tar.

In the final slide this is a brief summary of the tar recovery rates from exhaust gas using a standard carbureted engine, fuel was premium grade gasoline and I think you can read those numbers.

So the progress on the chemical and biological studies with the tar which has been recovered in these operations with leaded gasoline will be reported tomorrow morning by Dr. Hoffman and Wynder.

And in closing the first part of this paper I should like to emphasize again that our work today has been with only one engine and some experimentation has been carried out with pure hydrocarbon fuel and this work is continuing also -- exhaust gas has been sampled during the particular phases of the driving schedule -- and this work is also continuing.

The studies in the immediate future include the evaluation on the effect of air fuel ratio, engine load, engine type and several others.

Finally, I would like to point out the equipment has proved capable of providing detailed information as to the tar emission rate and a very convenient means for generating and collecting the relatively large quantities of tar required in the biological program.

The second part of the paper will be given by Mr. Colucci and will describe the air sampling equipment, or do you want questions on this part n

CHAIRMAN SAWICKI: Are there any questions or comments?

FROM THE FLOOR: I would like to ask Mr. Begeman if he could explain his choice of Soxhlet ? solid extracting the filter and the mixture of benzene methanol.

MR. BEGEMAN: I refer the answer to that to Dr. Hoffman.

DR. HOFFMAN: Before this was done we tried with the loaded filter various solvents and we came to the conclusion the best is benzene methanol four to one. This was tested before the final one was analyzed and we came to the conclusion for fuel this was the best.

CHAIRMAN SAWICKI: Any other questions?

MR. COMMINS: Commins, Dunn Laboratories, London, England. I would like to ask Dr. Begeman at what temperature is his collecting filter and whether he did attempt to assess what percentage of material went through the filter, if any?

MR. BEGEMAN: The temperature of the filter was 20 below room temperature, that is below about 70° as a maximum. I refer to the work, I believe it is by Dr. Napier of England who published that.

MR. COMMINS: We have done some work in England on that but what I wanted to know, the actual temperature of the filter to assess what the volitiles were due at any stage, assess the amount of material that may have passed through the filters.

MR. BEGEMAN: In a small scale experimentation we did. We found that the Number 50 FG medium recovered a minimum of 96 percent of that which was stopped on an absolute filter. We did not assess any loss of say pyrene or other compounds, and I think that's been answered in the first part of your question, the temperature was about 70° Fahrenheit on the filter.

MR. COMMINS: Thank you very much.

CHAIRMAN SAWICKI: Any other questions? I know our engineering department will be very interested in this research. Okay, thank you, Mr. Begeman.

Our next speaker, Mr. Colucci, I believe that's the correct pronunciation, received his Bachelor of Science Degree in Mechanical Engineering in 1958 from Michigan State University, his Master of Science Degree in Mechanical Engineering in 1959 from the California Institute of Technology. He has been employed at the General Motors Research Laboratories since July of 1959. His work has been primarily concerned with the design, construction and operation of the mobile air-sampling laboratory he will describe to you. Mr. Colucci.

MR. COLUCCI: In contrast to the tar collection from the engine where 24 grams are contained from a thousand cubit feet of gas it is necessary to sample over 3,000,000 cubit feet of air to obtain one gram of atmospheric tar. The basic design requirement of the General Motors air-sampling laboratory is that one gram of tar from the atmosphere in a period of approximately ten hours. The sampling rate required to collect one gram at ten hours depends on the concentration of tar in the atmosphere.

The urban concentration data for benzene-soluble material is determined by the National Air Sampling Network. These data show concentrations ranging from a half a microgram to 95 micrograms per cubic meter. Collection and extraction figures equivalent to those of the National Air Sampling Network, it will be necessary on the average to sample 3,120,000 cubic feet of air in order to recover one gram of tar.

Therefore to collect this one gram in ten hours a sampling rate of 5,000 cubic feet per minute is required. This rate is roughly 100 times greater than the rate of the so-called high volume samplers used by the National Air Sampling Network.

First slide, please. The entire sampling equipment here should be mobile so that it can be readily transported from one street level site to another. The sampling equipment should be easily transferable from the vehicle from which it will usually operate to building roof tops or other non-street level locations. And the last requirement is that the apparatus be capable of operating quietly.

The next slide shows three basic components necessary for sampling particulate matter from the atmosphere. They are an element for removing and retaining the particulates, a means for removing the air and a means of measuring the sampling rate. The filters especially in our application should have a high collection efficiency. It should contain a minimum amount of organic impurities. It should offer low resistance to air flow, it should have a low pressure build-up with particle deposition and it should have sufficient strength to withstand the pressure drop due to the air flow through it.

The choice of an all glass absolute filter medium for particulate is based on several factors. The first was a successful experience with such a paper as reported by the National Air Sampling Network. A second was the consideration of the size distribution for particulate matter dispersed in the atmosphere.

The next slide illustrates the size range of particles found in the atmosphere. It can be seen that particles as small as a thousandth of a micron are present in the atmosphere. It is of interest to note on this slide that the particle size range for lung damages the particles are between a quarter of a micron and teh microns and that the size range from formation lung retention is between one and three microns. In order to collect and retain particles as small as a hundredths of a micron the filter should have a high efficiency for these particles.

Filters having this capability are classified as absolute filters and are available in two general types. The first is a hundred percent all glass fiber. The second is a mixture of glass and asbestos fibers, a typical filter unit construction is shown in the next slide.

In this detail we can see that the filter paper is attached to the steel frame by means of a silicate cement. The paper is folded back and forth along the flow axis in order to get a large filtration area for a small filter frontal area. For instance, in this size filter with a frontal area of five square feet there is over 280 square feet of filtration surface. The pleats on the filter as shown here are supported by aluminum separators which also serve to channel the air flow through the filter. A single filter of the size shown on this slide, which is 24 inches by 30 inches, was selected in our sampling system, even though the pressure at a flow rate of 5000 cubic feet per minute would be much higher.

The alternative to this choice was the use of several filtering units and this was considered impractical. The pressure drop through the single filter is beyond the range of data available from the manufacturers.

Therefore, the final choice

of a specific filter was based upon minimizing both the pressure drop and the filter contaminates. The tests shown in the next slide were carried out as part of the filter evaluation program.

The first was a bench test to measure the paper flow resistance. The results of this test show that the resistance of the Flanders paper was less than that of any other papers tested.

The second test was a full scale pressure drop verus full flow test on 24-inch by 30-inch filter unit. Results of this test demonstrated that a flow rate of 4,000 cubic feet per minute the resistance of the Flanders unit was less than any of the other units tested.

Using the results of this test the calculated resistance for 24-inch by 30-inch Flanders unit at a flow rate of 5,000 cubic feet per minute would be four inches of water.

The third test involved filter media samples at 1000° Fahrenheit to determine how much removable impurities they contained. The results of this test showed that the all glass media contained less removable impurities than the combination glass-asbestos media.

The fourth and fifth tests were benzene extraction tests to determine the amount of organic material in the filter papers. The small scale test showed that the Flanders medium contained less extractable impurities than any of the other papers tested.

The large scale extraction of all the filter paper contained in a 24-inch by 30-inch Flanders unit gave a residue of 38/100 of a gram. This is less than ten percent of the average size of the samples collected to date.

This residue does not interfere with the analysis for polynuclear aromatic hydrocarbons.

Because the Flanders unit had both the lowest resistance to air flow and the least amount of organic material it was selected as the collection element in the particulate sampling system.

The basic flow requirements of a fan that is 5,000 cubic feet per minute air flow at five inches of water pressure were determined as the result of the filter unit pressure drop tests. These tests showed that the pressure drop of five inches of water would allow four inches of pressure drop through the filter and one inch through the rest of the flow system.

It is anticipated that permission to carry out sampling operations in residential areas would depend on the level of the sound generated.

Therefore, it is desirable to have the equipment operate quietly. Since the fan would be the primary source of noise it was desired to have this operate quietly also. A Joy central foil fan was purchased because it has the lowest operating noise level of any of the fans considered.

In order to determine the atmospheric concentrations of benzene-soluble material and carcinogenic materials the volume of air sampling must be known. This can be determined by integrating instantaneous sampling rates over the sampling time. A petot tube and an inclinometer are used with the air-sampling apparatus for measuring the air velocity and thus obtaining the instantaneous air flow rate.

This method was selected for the following reasons: It creates very little disturbance in the air stream and it does not increase the pressure drop in the system. And also it is a primary flow indicator. Therefore, it doesn't require calibration against a more fundamental method.

Upon selection of the basic components of the sampling apparatus the system ducting and electrical equipment were designed. This design was coordinated with changes incorporated in a standard Chevrolet step-van type truck so that it could accommodate the sampling apparatus.

The ducting design and truck body modifications were partially based on obtaining sound attenuation for noise that would be produced by the sampling equipment.

The next slide, please. The sampling components were arranged so that the air flow through them is in this order: First through the filter, then through the flow measuring section, and then through the fan. The fan that turns the air from a horizontal flow to a vertical flow where it is exhausted through a hole in the roof. The exhaust duct then turns the air 90° again so it is exhausted towards the front of the truck.

In our sampling operations it is anticipated that the air would be discharged in the same general direction as the wind to eliminate any possible air recirculation.

I should also point out at this time that during our sampling operations the truck remains stationary.

Two reasons prescribe the use of an electric motor as opposed to an internal combustion engine to drive the fan. The first is the absence of by-product particular matter in the case of electric motor. The second is the lower noise level of the electric motor.

A 7 1/2 horsepower motor drives the fan. Alternating current for the motor is supplied by the local utility company either by direct connection to overhead or underground wires or at a building outlet. Approximately 500 feet of four-wire heavy duty electrical cable are provided to allow the electrical connections to be made within that distance of the truck.

The truck in which the air-sampling equipment will be operated and transported was selected after the approximate weight and dimensions of the apparatus had been determined. A survey covering a variety of truck bodies resulted in a selection of a step-van body with cab over engine arrangement. This style of truck has also been popular with other air-sampling investigators. A view of the truck is shown in the next slide. As you can see it's nice bright colors and it can't be missed.

A rear view of the truck is shen in the next slide. This slide was taken at the General Motors Technical Center. The wide opening of the rear doors permits the removal of the apparatus in case we want to transport it to a building roof top for sampling.

The ducting part of the sampling system which you can see in this picture from here on can be removed by hand and transported fairly easily. The fan, motor and transformer and motor controls are mounted on a single removable wooden pallet so they can be taken out as a unit. This loaded pallet is shown in the next slide.

It weighs approximately 1,000 pounds and can be handled with a fork lift truck. In this slide you can see the arrangement of the various components, the motor, the fan, the incoming electrical switch and the motor controls, and down here in the lower corner you can see part of the wooden pallet.

The sampling equipment was designed to operate as quietly as possible. The sound developed by the apparatus and transmitted to the surrounding area is kept at a low level by the method shown in the next slide. These methods are: One, the use of flexible couplings at the fan inlet and outlet; two, independent rubber and sheer mountings for both the fan and the motor. This restricts the transmittance of vibrations from these sources. Three, is the sound observing lining in the system ducting. Four, is the use of gaskets between the duct sections to prevent transmission of vibrations along these sections. The fifth is the use of insulation in the truck body walls and roof. The sixth is a partition between the truck load space which contains the equipment and the driver's compartment. The seventh is a wooden floor over the steel sub floor in the load space. This wood floor also serves as a vibration and a sound-absorbing surface.

Upon completion of the design and construction of the air-sampling truck a program of air flow calibration and equipment familiarization was undertaken. Air flow calibrations were carried out by making three pitot tube traverses across the circular duct section. The traversing positions were horizontal, vertical and 45° with the horizontal. Three traverses were made to account for any non-uniformities in flow that might develop due to a transition from the rectangular filter section to a circular duct going into the fan.

For each traverse a velocity profile was plotted and an average velocity determined. The values obtained from each of the traverses were averaged to obtain an air velocity. This velocity when multiplied by the cross-sectional area of the depth of the measuring section which was 2.64 square feet gives the instantaneous air flow rate.

The next slide shows the results of the air flow calibrations. Several observations can be made from this table. First, a non-uniformity in flow through the circular duct section does exist. Consequently, results obtained from the averages of the three traverses are necessary to establish a correct calibration.

Second, a fixed relationship exists between the center line velocity and the average velocity for each traversing position. This relationship permits the air flow rate to be measured from the center line velocity once calibration is established. Quiet operation of the sampling apparatus was one of the criteria used in this design. To check out the effectiveness of sound attenuating measures sould level readings were made during air-sampling operations. These data are shown in the next slide.

I am sorry, I think we have the slides reversed here. The next slide probably is the right one. Yes, this is the right one.

I won't go through this table in much detail but the main observation from this is that the equipment does effectively attenuate the sound produced by the apparatus. One can notice if you are by the air-sampling truck when it is operating that there isn't any noticeable sound above the ambient conditions. The only place that you can notice any appreciable sound is right at the intake at the rear of the truck.

Preliminary sampling operations were carried out to obtain samples for developing procedures for removing the dirty paper from the filter and extracting the tar from the removed paper. Results obtained using a filter with a nominal air-sampling volume of 20,000,000 cubic feet are shown in the next slide.

These data indicate that the maximum air flow rate was never greater than 105 percent of the average or less than 93 percent of the average, and that the average air flow rate was 5,220 cubic feet per minute. With this type of flow variation the sample obtained should be truly representative of the material actually present in the atmosphere during the sampling period. This is a contrast to some of samples obtained by the National Air Sampling Network where in some instances a 50 percent decrease in sampling rate was observed from the start to the end of the sampling period.

After the particulate matter has been collected on the absolute filter the benzene-soluble portion is recovered. This process is carried out in the sequence shown in the next slide.

First, the filter paper is removed from the steel filter frame by cutting away two sides on a large band saw. The separators are removed from the paper and saved for a later washing in the extraction apparatus. When all of the separators are removed from the paper the paper is cut into the following portions, each of which is weighed. The first is one complete pleat which is approximately three square feet. This is used for lead determinations.

The second portion is

approximately one-third of a pleat and this is saved for visual inspection. The remainder of the paper is extracted in the 17 leader steam heated, stainless steel, Soxlet extraction apparatus — whew — previously shown by Mr. Begeman.

When extraction of both parts of the removed paper is completed the extract solution is drained and it is concentrated to a tar by two processes. Most of the solvent is stripped off by distillation and the final portions of solvent are removed by evaporation under vacuum using the apparatus shown to you by Mr. Begeman.

The amount of tar collected during the sampling period and its atmospheric concentrations are determined and shown in the next slide. The tar weight and the evaporation flask is corrected for three quantities. The first is the amount of organic impurities in your original unused filter paper. The second is the amount of tar that would be left on the paper that is not extracted. And the third is the amount of tar that is left on the paper still attached to the steel frame when it is cut away. The final tar weight is then divided by the total air flow volume to obtain the atmospheric tar concentration inmicrograms per cubic meter.

It has been shown that a correlation exists between traffic density and atmospheric concentrations of carbon monoxide and lead. Atmospheric concentrations of these two inorganic materials are measured in conjunction with the collection of particulate matter from the air. The concentrations measured are used to estimate the atmospheric dilution of exhaust gases. The dilution factor is to be used in determining the contribution by automobiles of carcinogenic material to the atmosphere according to the direct and indirect techniques previously described.

The continuous recording of the atmospheric carbon monoxide level in the particulate sample stream is obtained using a Beckman Model 21 long path non-dispersive infrared analyzer shown in the next slide. This analyzer has been modified for our use by removing the solid covers on both ends and replacing them with Plexiglas covers. These covers can be easily removed in case the operator has to get at the sample cells or electronic components. May I have the lights, please?

The air-sampling program that we will carry out calls for the collection of particulate matter in three cities, Detroit, New York and Los Angeles. Detroit has been chosen for preliminary operations to gain experience while the equipment is close to home. Sampling operations in New York City and Los Angeles will be carried out at several locations in each city. Each site will be sampled at three-month intervals for a period of at least one year. New York and Los Angeles were selected for the main program because of the climatic differences between both cities and because of the diverse air pollution problems that they are known to have.

Arrangements are presently being made for carrying out sampling in New York City. A mobile laboratory for collection of particulate matter from the atmosphere on an absolute filter has been built. Preliminaric operations have shown that the apparatus meets all of its designed criteria and provides the unique facility for the collection of atmostpheric particulate matter to be analyzed for polynuclear aromatic hydrocarbons.

Thank you.

CHAIRMAN SAWICKI: Any questions or comments? Please identify yourself.

MR. HOWERTON: Howerton, American Instrument Company, Silver Spring. Did you plot the velocity in the center on axis versus the off axis velocity?

MR. COLUCCI: The velocity profile consisted of making traverses across the duct section.

MR. HOWERTON: Across horizontal and vertical?

MR. COLUCCI: Yes.

MR. HOWERTON: And you got a curve, is it a parabolic curve?

MR. COLUCCI: Yes, fairly parabolic.

MR. HOWERTON: Both ways?

MR. COLUCCI: Yes. There are differences between the two and this is cause by the transition from the rectangular section to the circular section.

MR. HOWERTON: But that's minor?

MR. COLUCCI: Yes, small, very small.

MR. HOWERTON: Could you give me the velocity profile? I didn't understand it from your slide. Say one inch from edge of the tube, what would be the ratio of the velocity at the center?

MR. COLUCCI: Well, the velocity goes up very fast as you go away from the duct wall. It increases quite rapidly to almost the average velocity when you are probably an inch or two away from the wall.

MR. HOWERTON: But you don't have exact curves on that?

MR. COLUCCI: I have curves but I don't have them here. I could get them to you if you would like.

CHAIRMAN SAWICKI: Mr. Tabor?

MR. TABOR: One of the problems we have is the collection of a large sample that can be aliquoted for inter-laboratory comparisons. Is the deposit on the filter sufficiently uniform that it may be used for this purpose?

MR. COLUCCI: Well, the easiest way to get an aliquot is after the extraction of the sample.

MR. TABOR: Well, I am thinking of the original particulate matter itself.

MR. COLUCCI: You can do this by just removing a complete pleat.

MR. TABOR: Is this uniform?

MR. COLUCCI: Yes, from one pleat to the next they are identical, along the pleat from the front of the filter to the rear there is some difference. Most of the deposition is at the rear of the pleat.

MR. TABOR: Another question, after you are through in Los Angeles, can we borrow that truck?

MR. COLUCCI: No comment. Talk to my boss.

CHAIRMAN SAWICKI: Any other questions?

MR. STERN: I would just like to comment on the statement that was made that there is a 50 percent variation to be expected in National Air Sampling Network flow rates. This is not the norm and I don't want the impression to be retained that it is. It would be quite abnormal that this would occur and where we have very heavily filters in the National Air Sampling Network it would tend to show an extreme variation. We use the flow regulating devise to smooth that out.

MR. COLUCCI: I would like to make a comment. My data on this was obtained from the original publication from the National Air Sampling Network which covered data from 1953 to 1957 and in this publication it indicated that some of the samples obtained had this great discrepancy between flow rate but it didn't say that they all did and I hope I didn't imply this.

CHAIRMAN SAWICKI: Any other questions.

MR. HOFFMAN: Dietrich Hoffman, Sloan-Kettering Institute for Cancer Research, New York City. Mr. Colucci mentioned that our group asked for one gram of organic extractable matter from the filter for chemical analysis. This is not for chemical analysis alone, this is also for biological testing. We don't need one gram for a chemical analysis.

MR. COLUCCI: This, let me point out, is another reason we have made the apparatus as large as it is so we can also obtain samples from the atmosphere for use in biological experiments.

CHAIRMAN SAWICKI: Any other questions or comments?

Except for one item I want to point out that this afternoon after Dr. Neukomm's talk we will have a recess at approximately 2:30 and the meeting is adjourned until 1:45.

(Adjourned at 12:45 p. m.)

AFTERNOON SESSION 1:45 p.m.

CHAIRMAN SAWICKI: The meeting will come to order.

The first speaker for this session, I guess he will have the honor of being introduced three times in this meeting, so everybody ought to know him. He's had a Bachelor of Chemistry from DePaul University in 1943 a Ph.D. from the University of California, Berkeley. We have quite a few Universities of California and so we have to say where they are at. In 1950 he got his Ph.D. He worked in inorganic chemistry. He worked with Dr. Branch in spectrophotometric studies; he worked in sewage effluent reclamation; he did quite a bit of work on fluorescent spectroexamination, paper chromatograms, investigating polycyclic hydrocarbon composition of combustion products from gas, liquid, solid fuels, so he has done quite a bit of research.

I have one little comment here, and before he starts speaking, you might think it over. When a Californian breaths in your face he bows you over with the auto exhaust fumes. They've really got it over there, they're saturated. Dr. Thomas.

DR. THOMAS: Thank you, Dr. Sawicki. This is mainly the work of one of the bacteriologists in our group, and I being a chemist, I am likely to make a number of slip-ups in terminology, so you will have to bear with it.

The principal work that we are involved in in our research group is combustion and the analysis of the organic components found in polluted atmospheres. This work is an off-shoot of the analytical work because we have been able to separate over a hundred compounds in pure form, but these have been separated only in microgram and millimicrogram quantities.

Our approach to the problem is in contrast to the approach which has been proposed this morning, either you take and build larger devices for separating gross quantities of material from a polluted atmosphere, or else you take and use conventional techniques if your budget doesn't allow the building of these trucks and so forth, and try to develop a technique that will allow you to use a test animal proportionate to the amount of test material.

For injection and ingestion you use relatively large quantities of material in the test animals, a mouse or any of the other test animals.

This test was developed using the microgram quantities of material on bacteria. The order of magnitude being a quarter of a microgram per CC of test material.

I should mention that the assay technique has been performed or has been developed using only four compounds in the scale of carcinogenicity, two compounds at one end of the scale and two compounds at the other end of the scale, the compound in question being benzo(a)pyrene , 3,4-benzpyrene and methyl-cholanthrene at one end of the spectrum and at the other end of the spectrum phenanthrene and anthracene, and the characterisation of these compounds or the toxic, nontoxic or carcinogenicity was to try to use morphology and biochemical results to characterise the compounds, but I should emphasise that it has only been done for four compounds. Actually it has been tried on more than four, but I am only reporting the results of the four compounds mentioned.

May I have the first slide, please? The organism that was used with bacillus megaterium after screening a number of organisms this was found to be the most reliable organism because of its stability and the rapidity with which it reacted under the various conditions.

Now, for the morphology studies the bacteria were grown in heart infusion broth to which was added the hydrocarbons as mentioned a quarter of a microgram per millimeter of broth.

Now, these pictures here show the control and also the cells that are grown in the presence of anthracene and phenanthrene. There is nothing spectacular about these cells, they stain, they have a rather uniform appearance.

May I have the next slide, please? Now, these are the same cells grown in the presence of 3,4-benzpyrene. You will notice that there is a long -- Can this be focused in any better? The long cell which has failed to divide is reticulation in the cell and there are a number of abnormal growths that are noted throughout the pattern.

May I have the next slide, please? Again can this be focused a little better or is it possible to turn the light out in the rear of the room and I think you can pick out the abnormal cells. I wonder under these conditions if you would mind throwing back 1 and 2 also so that you can see the difference between the normal and the abnormal cells. This is in 3,4-benzpyrene and there are a couple that have been marked out. Not all of them are abnormal, but there are a goodly number that are abnormal. Now the normal and the control and those grown in the presence of anthracene and phenanthrene appear on this slide.

May I have the slide 3-A then? That's right, it was coming up right. Now, this is the cell in which 3,4-benzpyrene was introduced into the media.. This was taken on a high dry using ultraviolet light as a source. It took twenty minutes to take this picture, but this indicates the penetration of the 3,4-benzpyrene into the cells and you see a number of the abnormal cells. Only the abnormal cells show in this picture, so you have the giant cell or the undivided cell. I can't show you a picture of the control or the anthracene or phenanthrene because under the similar conditions the field is completely dark. There is no penetration of the so-called noncarcinogenic material into the cell. Only in the presence of the two carcinogenic material was there a penetration into the cell and these are actually centers within the cell of the carcinogenic material.

May I have the next slide, please? Now, the biochemical studies, the bacteria were grown in a chemically defined media using glucose as the only carbon source. There is no difference in the morphology when the cells are grown in this chemically defined media. The one difference that is significant as indicated on this slide, the difference in the growth rate.

You will notice that there is a lag in the chemically defined media, the lag being for the 3,4-benzpyrene and the cholanthrene compared to the heart infusion broth which there was no lag whatsoever.

May I have the next slide, please? This is the infrared spectrum of lipid extract after twenty-four hours incubation and as indicated, A is the control, B is the phenanthrene, the noncarcinogenic compound, C is methyl-cholanthrene, and you will notice the characteristic peaks that appear on the infrared spectrum as circles.

Now, may I have the next slide, please? The same inflection can be observed in a forty-eight hour culture appearing at the same wave lengths that are indicated and you will notice there is a lack in the control or in the presence of phenanthrene.

May I have the next slide, please? This slide indicates the total lipid material produced. You will notice that the hydrocarbons, all hydrocarbons enhance the lipid production. The control 7.3 phenanthrene and anthracene there is an enhancement, but the carcinogenic hydrocarbons, the last two enhanced to a greater extent than do the phenanthrene or anthracene. Forty-eight hours there is still a noted difference, but at the end of seventy-two hours they all tend to catch up. The same points might be noted with the lactic acid production. All hydrocarbons enhance the lactic acid production, the hydrocarbons that are conventionally classed as strongcarcinogenic enhance it to a greater degree in the twenty-four hour culture and the forty-eight hour culture, but there is a catching up in the seventy-two hour culture.

May I have the last slide, please? This slide indicates the effect of the polycyclic aromatic hydrocarbons on the methylene through reductase activity, and it's an indication of the ensyme activity and you will notice that the minutes for the complete disappearance of a blue color and for the phenanthrene and anthracene it's a relatively longer period of time compared to benzo(a)pyrene and methyl-cholanthrene,and for the forty-eight hour culture you will still notice the trend is there, and for the seventy-two hour culture there is the trend still being maintained.

These are preliminary data but they look very promising and I should mention that work is being carried out now screening other bacteria, looking for more extensive biochemical tests and also the main point we are starting to screen a larger variety of compounds and at this last point I should mention that the assay still looks very promising.

Before I throw myself open for questions I should remind you that I am not the bacteriologist responsible for the work.

CHAIRMAN SAWICKI: Are there any questions or comments? Don't forget to identify yourself. That will help.

DR. KATZ: Dr. Katz, Department of National Health & Welfare, Ottawa, Canada. I would like to say first of all as a comment that I think this work of Dr. Thomas fills an urgent need which has been evident for very many years in the bioassay of polycyclic hydrocarbons the fact that by using small quantities in the range of a quarter of a microgram per ml of solution, one can obtain within twenty-four hours definitive tests for carcinogenic potency is a remarkable thing and I think that if the contents of Dr. Thomas's paper become available to us as soon as possible, it will enhance and stimulate future work. I would like to ask one question. Have you tried other organisms and what effects have the polycyclics had on other organisms and also have you been able to try out, for instance, chrysene or coronene or some of the other doubtful carcinogenic hydrocarbons in the case of your enzyme tests on the growth rate of the cultures?

DR. THOMAS: There have been a number of organisms tried and I don't want to say similar results, comparable results can be obtained. We were looking for an organism that was very stable, that would not undergo mutation to any great extent, and the bacillus megaterium was the best that we could find, although we don't feel that it is the ultimate. If we look long enough we will probably find an organism that is better. Right now we are satisfied with it and going on with the work, but as a side line we are looking at other organisms.

Now, as far as testing other materials, we have tested a number of other compounds which have been found in polluted atmospheres and have been identified and are conventionally classed as either being carcinogenic or noncarcinogenic and it's probably this classification is a degree. There are other people that have stated that the compounds that are normally classed as noncarcinogenic, the tests being based on whether a tumor can be induced in the mouse, the mouse died a normal death before the material had a chance to become operative, and in this particular test it is possible that there are indications that a compound which has previously been classed as noncarcinogenic, we might extend the scale and say that compounds previously classed as noncarcinogenic might become now weakly carcinogenic. That type of spectrum might be developed.

DR. LYONS: Dr. Lyons, New York. I want to disagree that these tests have any definitive nature at all that the range is too small. For proper tests of this sort and for anti-carcinogenic studies we should strive to test compounds which are sterically and electronically as close as possible. A good range of compounds to test would be the methylated benzanthracene. In that theory you have a wide range, you have electronic similarity and steric similarity and a wide range of carcinogenic activity within that family of compounds. There are three ring compounds, there are five ring compounds and that sort of thing.

DR. THOMAS: Well, this probably would be incorporated into the program. We realise this particular point. Initially we wanted to look at compounds which might be found in the polluted atmosphere to see if there was any possibility and as I mentioned earlier the fact that we can't find the entire array of methylated compounds that you indicated in the polluted atmosphere, it doesn't rule out the test effect. It should be related to the initial work that was done on the mouse with the same type of compound. It should be extrapolated out into the work on the bacteria. We just haven't done it. We have only gotten into the work in this sort of a preliminary report.

CHAIRMAN SAWICKI: Any other questions?

DR. FALK: Dr. Falk, California. I presume you are all aware of the fact that there is another test being developed on paramecium, and this test completely depends on light in activating the organism and killing them, so that I wanted to ask the question whether in your studies you eliminate light while these organisms are growing and while you keep them alive or whether they are exposed to light in which case your test might get somewhat similar to the one on paramecium which is giving similar results.

DR. THOMAS: We are aware of this other test. Ours, of course, is in the bacterial --

DR. FALK: Is yours in darkness?

DR. THOMAS: No, it isn't. We don't make any effort to keep it in the dark. Our cultures are grown in the shake incubator which has a glass window on the top, but we make no effort to cut out the light.

CHAIRMAN SAWICKI: Any questions?

MR. BEGEMAN: Begeman, General Motors, Detroit. I think I must have missed a point in connection with one of your slides. I think you identified it as 3-A which you said about twenty minutes was required for the exposure. I wasn't sure what we were looking at. Is this a radioactive view or fluorescent?

DR. THOMAS: That was a fluorescent. We were exciting the 3,4-benzpyrene which is fluorescently invisible when excited with ultraviolet light. If you look at the cells under the microscope with ultraviolet light, the source, you cannot detect any fluorescence,, but when you use the camera instead of your eye for a long period of time the small amount of fluorescent energy burns into the film and you get the picture that you saw in the screen. It took twenty minutes for that, so it's fluorescense that you are looking at.

DR. VAN DUUREN: Van Duuren, New York. I would like to ask, Dr. Thomas, to what do you ascribe the small differences, the three peaks in the infrared of the lipid group in which methyl-cholanthrene has been used?

DR. THOMAS: Well, two of them are out in the fingerprint region, so I don't want to make any guess there. The third one, if I am not mistaken, is in the carbonyl region. I haven't looked at them outside of on the slide here in the last month, so I will withhold really any statement. I would have to look at them closely again. I know that we have discussed this in the lab and I have forgotten what our conclusions were.

CHAIRMAN SAWICKI: Any other questions?

Thank you, Dr. Thomas.

Our next speaker is not with us. He could not come. Dr. S. Neukomm, who obtained his M.D. in 1943 from the University of Lausanne, worked for a year at the Institute of Radium in Paris, head of the department of experimental research of the Institute from 1951; his main field of investigation has been the study of carcinogenic substances and their relationship with steroid hormones. He has collaborated since 1954 with Dr. Bonnet on the study of carcinogenic and co-carcinogenic substances in tobacco smoke. He is a professor of cancerology, University of Lausanne. I have heard him talk before on the Newt and I think it's fairly interesting and it sort of follows in line with what has been given, and I will read his paper.

The Newt test in relation with investigation on carcinogenic and co-carcinogenic substances. Investigation of substances in the environment, for example, polluted air, tobacco smoke of carcinogenic activity has still one important difficulty: the very long period of time necessary for tumors to appear in treated animals. As you all know it takes sometimes a lifetime of a mouse or rat, sometimes six months. That's a long experiment. As he says this is especially true of mice and rats which are considered specially capable of a response to carcinogenic substances.

The purpose of the present paper is to show that a satisfactory solution has been found to reduce considerably the length of the latent period for induced tumors, produced by certain carcinogenic and co-carcinogeni substances, by utilising a type of batrachian, the Newt. This is a small salamander. It belongs to the frog family.

Technically speaking the Newt test is very easy to do. The product in oily solution is injected under the skin at the tail of the animal. Six to eighteen days later the tail of the animal is amputated and histological preparations are made of the injected region.

The reaction of the epidermis is obtained according to two different processes considered separately. Hyperplasia on the one hand, that's excessive cell growth, and infiltration on the other. When infiltration is accompanied by hyperplasia in the injected region, thetest is positive. Infiltration without hyperplasia is considered as a negative test as well as hyperplasia alone.

During the first series of studies, the reaction provoked by certain aromatic hydrocarbons such as benzene, naphthalene, acid naphthene, anthracene, pyrene, chrysene, 1,2-benzanthracene, benzpyrene, dibenzanthracene and 20 methylcholanthrene was studied. Chrysene, benzanthracene, benzpyrene, and methylcholanthrene gave a positive reaction while the other substances did not give such a reaction.

Tests for these particular hydrocarbons, a Newt test can be substituted for the known reactions of the mammalian skin. On the other hand, we have also tested nitrogenis high carcinogens, in particular B-napthalene, acetylaminofluorine, 2-aminofluorine, and butter yellow. We observed that B-napthalene, acetylaminofluorine give a positive reaction, while 2-aminofluorine and butter yellow give a negative reaction. These are both carcinogens when it comes to application to the mouse.

These substances do not, therefore, produce exactly the same effects on mamals as on the Newt, and let me point out briefly that the Newt reacts to very weak concentration of 3,4-benzpyrene, 10 to the minus 5 grams per leader. Dibenzanthracene 10 to the minus 7 grams per leader, and 20-methyl-cholanthrene 10 to the minus 9 grams per leader. These are very dilute sollutions.

The injection of amounts less than a microgram is therefore still sufficient to give a positive reaction.

The Newt test can be used to demonstrate the carcinogenicity of aliphatic hydrocarbons as well as polycyclic hydrocarbons. The systematic utilisation of the Newt test and these studies on the chemical composition of tobacco smoke allowed us to find out in 1956 the carcinogenic and co-carcinogenic power of the following compounds: 3,4-benspyrene on the Newt, 3 plus on the mouse, three plus; 3,4,9,10-dibenspyrene, three plus on the Newt, plus 1 on the mouse; 1,2-bensanthracene, plus 2 on the Newt, plus minus on the mouse; which means it could be weakly carcinogenic. 5,6-cyclopenteno, 1,2-benzanthracene, plus 2 on the Newt, plus 1 on the mouse; 6,7-cyclopenteno, 1,2-bensanthracene, plus 2 on the Newt and plus minus on the mouse, and the aliphatic hydrocarbons, paraffinics, plus 2 on the Newt and plus minus on the mouse.

All compounds which were isolated in chemical studies by Dr. J. Bonnet were tested on the Newt. Substances which gave a positive response were then tested on the mouse.

In conclusion the Newt test was found to be extremely useful for the investigation and the identification of carcinogenic and co-carcinogenic compounds contained in tobacco smoke. Its utilisation should be equally useful for investigation on the air pollution.

That concludes the talk. Any questions or comments? Dr. Thomas?

DR. THOMAS: Thomas, University of California, these tests should be tried eventually with substances of similar geometry and also methylated compounds,and I think Dr. Thomas' statement that they are interested in the material in air pollution and do not find the methylated compounds to any large extent, so they may be able to apply it to air pollution.

Any other comments?

Well, in that case we are adjourned for recess and we come back at 2:45.

(Short recess.)

CHAIRMAN SAWICKI: Our first speaker for this part of the session is Dr. William Payne, who obtained his B.S.E. in civil engineering in 1935, University of Virginia, M.S.E. in Sanitary engineering in 1947 at the University of Michigan, and Masters of Public Health in 1956, University of Pittsburgh, and his Doctors of Science in 1959 at the University of Pittsburgh. He has worked on carcinogenous dechromium compounds in rats, carcinogenic bioassays in air pollutants, and he will now discuss his paper on special requirements for bioassay for carcinogenicity. Dr. Payne.

DR. PAYNE: I hope these somewhat general remarks will serve to stimulate a little further the work such as the type described this morning by the two gentlemen from General Motors as well as more of the possible bioassay methods that have been described this afternoon. Such methods we hope

Berkeley. Newts that my kids play with, the tail falls off all the time and they grow a new tail. Does the test hold for the new tail as well as the old tail?

CHAIRMAN SAWICKI: Did you say nude or Newt tail? I am not sure here. I don't know.

DR. THOMAS: New Newt tail.

CHAIRMAN SAWICKI: I don't know. The Newt is a very interesting animal in that respect. Kids can pull it apart and the pieces walk off. I don't know. Dr. Lyons, do you have a comment? Dr. Hoffmann?

DR. HOFFMANN: Dietrick Hoffmann, Sloan Kettering Institute, New York City. Only for the record, there exists development in this country and other short term ~~tests further~~ developed by Bach, and there were tested the destruction of the basic gland from mouse skin of various fraction; also tobacco smoke condensate as well as polycyclic hydrocarbon, but in difference to the Neukomm-Bonnet group, and ~~others~~ included in this group, don't make any final statement, that since there is a basic gland destruction, therefore, this material is carcinogenic, but the indication is that long-scale carcinogenic tests should be done, but no definite answers are drawn from this test. Thank you.

CHAIRMAN SAWICKI: Any other questions?

Well, I think that Dr. Lyons' comments hold and Dr. Thomas' both. Dr. Lyons that might be of considerable assistance in carcinogenic screening methods.

The objective of this seminar or symposium is the exchange of information on methods of sampling, separation and analysis of air pollutants and to compare the data for organic substances suspected of playing a role in the carcinogenic process.

Since bioassay for carcinogenicity may be considered a special kind of analysis, a review of the results of the somewhat limited investigations that have been carried out on the carcinogenicity of atmospheric pollutants seems appropriate. At the same time the limitations of present bioassay methods as well as that of the materials available for testing should be recognized.

Finally, the special requirements for the collection, separation and analysis imposed by such testing should be studied with a view to improving and developing these techniques and thereby increase the value of bioassays and promote the development of better ways of evaluating carcinogenicity.

The study of the carcinogenicity of atmospheric pollutants by the administration of dusts from the air is not a recent innovation. In 1922 Passey reported the production of malignant tumors in mice by the cutaneous application of an extract of household chimney soot, a major source of air pollution in England at that time. Campbell in 1934 published the results obtained in the exposure of mice to the inhalation of dust obtained from tarred roads. Compared to the control group there was a ten-fold increase in the number of primary lung tumors among the animals exposed to the dust. Cancer of the skin developed in seventy percent of the exposed mice surviving long enough.

Campbell also exposed mice by skin painting to tar obtained from road dust and chimney soot and found both to contain potent carcinogens.

Siegal and Shear in 1938 collected dust by the "soot-fall" method at 150 locations in New York City and administered extracts to mice by subcutaneous injection. No tumors were found at the site of injection an no noteworthy increase was found in the number or size of pulmonary tumors.

McDonald and Woodhouse xposed mice by inhalation and skin painting to atmospheric dust from two sources, the air purifying screens of the Birmingham General Hospital and flat-roofed buildings in entral Birmingham. The incidence of lung edenomas in the mice exposed to the dust both by inhalation and painting was somewhat higher than in the control groups.

In 1942 Leiter, Shimkin and Shear published the results of the examination of nine specimens of dust from the atmosphere for carcinogenic potency. Dusts were obtained from several cities by various methods including electrostatic precipitation, filtration through cloth and labyrinth precipitation. Unextracted dust as well as benzene and ethyl ether extracts were injected subcutaneously in the right axilla of mice. No tumors were produced by the unextracted dust, while sarcomas were observed in 18 of an effective total of 291 mice which received a single injection of the tar extracted from the dust.

Because of objections to the collection methods employed in the previous studies Leiter and Shear developed a large-capacity collector utilizing filter paper to obtain atmospheric dust samples from five industrial cities. Following the subcutaneous injection of benzene extracts of the dust tumors developed at the site of injection in 30 of 372 mice or 8 percent of those surviving 5 months or longer. The differences between the several cities were not conclusive.

Clemo, Miller and Fybus tested three chemical fractions separated from industrial smoke in an English city by skin painting of mice. Two of the fractions produced malignant tumors and papillomata.

In a study of material collected from the atmosphere of Los Angeles, Kotin, Falk, Mader and Thomas supplemented chemical studies with biological tests for carcinogenicity. A benzene solution of the extract from natural smog was applied to the skin of mice. At the time of appearance of the first tumor 31 of 76 mice were surviving; 13 of these developed tumors.

In addition to the biological testing of smoke and dust from the atmosphere a number of investigators have studied these materials to identify and isolate known or suspected carcinogens while others have investigated the carcinogenicity of combustion products, industrial wastes and other materials which are discharged into urban atmospheres.

Utilizing materials collected, fractionated and analyzed by this Sanitary Engineering Center, Hueper, Kotin, Payne and Falk in 1957 began the carcinogenic bioassay of four organic fractions of the particulate phase samples of air pollutants collected monthly from eight American cities. Although the conventional subcutaneous injection and skin painting techniques were employed, the experiment was designed with several specific objectives other than the demonstration of the presence of carcinogens in the air of the eight cities:

1. to determine whether a correlation exists between the carcinogenicity to experimental animals of the materials collected from the air and the relative incidence of cancer of specific sites in the human population.

2. to compare the tumor yield of the crude benzol extract and the aromatic fraction with the benzo(a)pyrene content of each sample and to determine whether the presence of other chemicals might influence the tumor yield either positively or negatively.

3. to compare the tumor yield of the fractions with the yeild obtained by the administration of specified doses of benzo(a)pyrene under similar conditions.

4. to compare the carcinogenic potency of the four fractions and thus evaluate the relative importance of the groups of compounds.

5. to evaluate present bioassay methods and to provide a rational basis for planning future studies.

Although the precedures and the preliminary results were presented by Hueper at the 53rd Annual Meeting of the Air Pollution Control Association held in Cincinnati on May 25, 1960, a brief review of the results will be presented here. The final results will be published in more detail later.

Can I have the first table, please? Of the 2618 mice, exclusive of controls, included in the experiment seventy developed cancers at the site of administration. I would like to point out that the benzol subcutaneous, the aromatic subcutaneous, the work was done at the Cancer Institute; the oxygenated, both the subcutaneous and the painting and the aliphatic fraction, subcutaneous and painting were done at the University of Southern California.

As I said the material was collected and processed by the sampling network here and the processing of the material was done here at the Taft Engineering Center.

You will notice that all fractions produced some tumors and some tumors produced in all cities, although we almost missed Los Angeles, a single tumor from all of them.

I would like to point out too that there were equal number of mice in the groups for each city, so percentagewise the actual number of tumors would be comparable for the various cities.

No, similar tumors occurred among the controls.

Table 2, please. Table 2 gives a comparison of the number of tumors produced, the total was produced by the benzol fraction and the aromatic fraction shown with the benzpyrene content of the benzol extract as determined by Sawicki and others here at the Center.

Could I have that one back just a moment, please? The correlation between the number of tumors produced by the bensol fraction and the benspyrene content seems to be fairly good for Detroit, Birmingham, New Orleans and Los Angeles, but not for Cincinnati. The other cities sort of in between.

The next one, please.

The next table is similar except that we have a comparison of the amount of benspyrene per injection of bensol extract, and incidentally, if the tumor yield were due entirely to the benspyrene, theoretically at least the amount -- the yield should have been the same from the aromatic fraction since the aromatic fraction was dosed in a proportionate amount that would equal the amount of aromatic extract that would be obtained from the four milligrams which was the dose of the bensol fraction.

Figure 1, please. To explain the comparison with the standardized dose, the total amount of benspyrene was in an experiment which a given amount of benspyrene were administered to groups of 72 animals in each group. In one case the total amount was given as a single dose and in the others the same amount was divided in 12 monthly doses, so to compare the yield from a given city, if we had, for example, a 20 percent yield of tumors, we could determine what under these conditions the same dose of benspyrene would have given that same response.

Could I have the Table 3

back again? You can see that the correlation between the equivalent dose of benspyrene and in general the others is in at least the same order of magnitude.

Table 4, please. The age-adjusted primary lung cancer mortality ratio for white males as published by Manos, 1949 to '51 for each of the eight cities and the number of mice with tumors at the site of application are given in Table 4. The total number of mice with tumors is in agreement with the observed lung cancer ratio in only one city, Birmingham. This ratio, incidentally, is the ratio of the lung cancer in this case for white males as compared to the United States as a whole, so all these cities are above or slightly above the average for the United States.

May I have the lights back on, please?

Technical difficulties delayed the administration of the entire particulate phase of the air pollutants collected from the cities under study, but this phase of the experiment as well as administration to mice by other routes including intratracheal injection have been undertaken at the National Cancer Institute. Preliminary results indicate that survival and tumor yield will not be very satisfactory. It should be noted that exposure by inhalation has not been undertaken because of the technical difficulties involved and the lack of success in producing primary lung carcinomas in experimental animals by the inhalation of other materials, some of which are known to be carcinogenic when administered by other routes.

The bioassay experiment for the eight cities, a joint undertaking of the Air Pollution Medical Program of the Public Health Service, this Center, the University of Southern California and the National Cancer Institute, illustrates and emphasizes the limitations of present carcinogenic bioassay techniques and of methods for collecting and recovering materials for such bioassays.

First only a portion of the pollutants present in the air being sampled is collected. Except for whatever may be adsorbed by either the collecting medium or by the material that has been collected the gaseous phase is absent from the sample. Generally only the organic fraction, or a part of it, is recovered from the collected particulate matter. The omission of gases and constituents which are not soluble in the agent used for extraction may result in the loss of a carcinogen or carcinogens or a substance which plays a contributory role in the carcinogenesis process. The effect of collection and processing methods on carcinogens that may be present in the air is not known fully. Prolonged sampling periods, for example, may result in the oxidation of a carcinogen to a compound that is not cancer-producing. Some of the apparent discrepancies in the previous comparison of the number of tumors produced by the aromatic fraction with those produced by a standard benzpyrene solution may be the result of such an action, even though extraction and fractionation were performed with utmost care to minimize chemical changes.

Another important consideration is the change that may occur between the time pollutant materials enter the air and the time they are collected. Climatic conditions may modify the composition of materials present in the atmosphere either to reduce or enhance tumor-producing properties.

Reference to the extensive research that has been conducted on the components of the Los Angeles atmosphere will give some suggestion of the potential changes that may take place after combustion products, industrial wastes or other pollutants enter the air. Similar or even more drastic chemical changes may be brought about by the interaction of compounds originating from different sources resulting in an increase in carcinogenicity. Conversely interaction may result in reducing the carcinogenicity of samples administered to animals. As an illustration, Payne found that increasing amounts of chromates administered with a constant dose of benzo(a)pyrene resulted in a decreasing number of tumors in mice. Chemicals, metals for example, that may be present in the air in very low concentrations may serve as catalysts producing reactions that would not occur otherwise. On the other hand transient compounds that may be present in a plant effluent may no longer be present when the material is analyzed or administered to animals.

The number and location of air sampling stations within a given area in relations to sources of pollution, prevailing meteorology, topography and population distribution are of utmost importance in any study of the relation of air pollution to health effects.

In the studies that have been described it must be recognised that the materials tested in animals may not be representative of the material actually present in the air from which the sample was taken; if the sources of pollution are numerous in the area, the air from which the sample is withdrawn may not contain the contaminants in the proportion that they exist in the area as a whole. If sampling stations are not sufficient in number or if they are located without regard to prevailing wind direction, a carcinogen arising from a limited geographical area may be omitted from the test material. In the comparison of the results of either chemical analysis or bioassays with observed cancer rates in the population of the city under study correlation should not be expected if the population distribution in the city is such that large segments are not exposed to the air from which samples are taken. These considerations would indicate that investigations should be planned in which smaller geographical areas are studied more intensively.

I notice that the General Motors plant has apparently taken that approach in concentrating a sampling machine in different locations and in longer periods within the city.

In summary, to obtain better results from bioassays a number of aspects of sampling as well as bioassay methods themselves need to be developed and improved. All substances comprising the air pollutant material should be collected and recovered in a form that can be administered readily to animals preferably without the use of a vehicle. The material should include not only organics but minerals and the gaseous phase. The sampling and collecting apparatus should be capable of collecting the material in a short time and in a manner that will minimize changes in the state of the substances and interaction between components. Taking into consideration meteorology, topography and sources, sampling stations should be located in sufficient number and operated in a manner to insure that the material administered to animals is representative of the air contaminants in the area under study. Materials recovered by sampling should be in relatively large quantities sufficient for administration to a large number of animals over a long period using, preferably more than one species of animal and more than one route of administration. As methods for sampling, separation and analysis are improved, techniques for carcinogenic bioassay must be modified and refined and new approaches developed.

CHAIRMAN SAWICKI: Are there any questions or comments? Dr. Katz?

DR. KATZ: I think Dr. Payme has touched on points which are of great importance to workers in this field, the fact that the samples required for bioassay are of an entirely different order of magnitude than samples required for chemical work and also he has raised the matter of the stability of the samples and the possibility that some of the material which one intends to collect may not be there at all, and we have encountered this same problem in connection with the fact that such things as anthracone, naphthalene and anthracene may be mysteriously absent from the polycyclic fraction, and I ask the question why should that be so if the sample is properly collected? You should be able to get a cut all the way from naphthalene right up to coronene, and so on, and everything in between, and yet in other cases where you specifically go to hunt for naphthalene and anthracene and so on, in these lower polycyclics that are extremely volatile or at lower temperatures, you can find them if you use solvent instead of the particulate filter technique, so I think he has really hit the nail on the head in stressing the fact that one needs better samples than merely those collected on particulate filters. I would like to hear some discussion on that point.

CHAIRMAN SAWICKI: Any other questions? Dr. Van Duuren?

DR. VAN DUUREN: Van Duuren, New York University. Dr. Payne, one of the tables shown on the slide you had listed carcinogenic materials and reported on the carcinogenicity. Could you define these materials chemically?

DR. PAYNE: I wonder, since that was part of the Southern California work, they handled that material, I wonder if maybe Dr. Falk would like to comment on that.

DR. FALK: I can pass it on to the next group who actually made that material. It is a brown oily fraction which is called actually oxygenated neutral material and it was obtained mainly by having the fractions that wanted to come off the column of alumina removed and then taken to the end before the napthas and the phenyls came out, so the separation that was obtained -- I didn't do it. I just got those fractions myself -- was that the aliphatic would be removed first by alumina chromatography, followed by the aromatics which were then removed, followed by the oxygenated neutral material, and somewhere in the end the phenyls and the acetics were either retained, tested or sold otherwise.

DR. VAN DUUREN: If I can take Dr. Sawicki's place, maybe I could ask him if he would like to add anything to that.

CHAIRMAN SAWICKI: Oh, I am good at passing the buck too. Mr. Tabor?

MR. TABOR: I think Dr. Falk pretty well covered the situation, although I might mention just to briefly outline the procedure. We first removed the acid material and the basic material. The third fraction, the neutrals was then chromatographed so we eluted the aliphatic hydrocarbon, then we eluted the aromatic hydrocarbons and we kicked everything else off we could get and that's the package we sent to you, Doctor. We call that, for lack of a better name, the oxygenated neutrals. They were neutral chemically and they certainly did contain oxygen, and that's about as vague as you could make it.

CHAIRMAN SAWICKI: Any other questions? Dr. Van Duuren?

DR. VAN DUUREN: My interest, of course, in this is because of the reported carcinogenicity of epoxides, hydroperoxides, and related material, and I was wondering whether any of these type of compounds would be in that particular fraction.

CHAIRMAN SAWICKI: Dr. Hoffmann?

DR. HOFFMANN: May I ask a question? Dr. Van Duuren, wouldn't you expect in the fraction after the polynuclear aromatic hydrocarbons some of the dibenzacarbazoles which you so well identified, couldn't they be in this oxygenated fraction?

DR. VAN DUUREN: According to Dr. Falk this is a neutral fraction and they are in the basic fraction.

CHAIRMAN SAWICKI: This is a neutral fraction. Yes, you could find a dibenzacarbazole, true. Dibenzacarbazoles you would expect to come after in a neutral fraction.

DR. KATZ: I would like to ask a question about the Los Angeles results. Now, one would expect that with the three million or more motor vehicles operating in the Los Angeles area and the evidence for the carcinogenic hydrocarbons in automobile exhaust gas and so on, one would expect greater potential carcinogenicity for the Los Angeles atmosphere than is shown in the data reported by Hueper and his co-workers and yet Los Angeles is virtually the last on the list. Is there any explanation for this? I refer this to Dr. Payne or Dr. Sawicki.

DR. PAYNE: The best thing I think I can do is to say that I have no good explanation. We were certainly surprised that such a low yield of only one tumor out of all the groups and that tumor, incidentally, occurred in a Los Angeles mouse, not a Bethesda mouse.

DR. KATZ: I think, Dr. Sawicki, I think you have some suggested explanation for this, have you?

CHAIRMAN SAWICKI: Well, I think now Dr. -- well, passing the buck -- but they're looking for carcinogens in auto exhaust and they have studied ozonides in auto exhaust and I think they expect to find some carcinogens in the aliphatic fraction, something like that, so maybe Dr. Falk could make a few comments.

DR. FALK: Falk,S.C. The problem that we had in Los Angeles was that first of all the quantity of benzpyrene in the atmosphere is very small and due to the fact that we do not use coal or otherwise we have got the cleanest people in the world, there is no benzpyrene to speak of compared to Chicago, New York and other coal-burning cities. There is plenty of benzpyrene in the automobile exhaust. That's to contradict my previous statement, but there isn't any of this tremendous amount of benzpyrene as we find in other areas, however, we found another carcinogenic agent, namely, epoxide, and I shall show a slide, if I get there, where the probability of forming epoxides in automobile exhaust is actually quantitative. I will show it to you later on. The low incidence of tumors in the Los Angeles area in this mouse study which will be some year contradicted by the actual physiological data obtained in Los Angeles in 1975 or so is partly applicable, I feel, on the changes non-specific in nature due to the breathing of this type of air pollution it is not necessarily highly carcinogenic, in preparing ground for a carcinogenic response due to other chemicals which are present which will be much higher than other areas just because the conditions are prepared better for cancer and I shall spend at least ten minutes on this aspect twenty minutes from now.

CHAIRMAN SAWICKI: Any other questions?
Dr. Badger?

DR. BADGER: Dr. Badger, Australia. If I may return to Dr. Van Duuren's question about the epoxides. Would it not be the case that the epoxides, if present, would come off the hydrocarbons, the aliphatic hydrocarbon fraction and ask whether there was any oxygen in this fraction.

CHAIRMAN SAWICKI: Did you ask Dr. Van Duuren? Oh, Mr. Tabor.

MR. TABOR: We did not analyse any samples to see if oxygen was present. Most of our measurements were limited to infrared examination. Now, the aliphatic extracts that we did send out for experimental purposes showed remarkably true pictures of aliphatic hydrocarbons. We did not find any indication of anything other than the pure hydrocarbon bands, however, this does not rule out the possibility of a slight amount of oxygen being present.

DR. BADGER: Thank you.

CHAIRMAN SAWICKI: I would like to make one comment here. Of course if epoxides were present the infrared probably would not show up, if it did it would be present in very small amounts, and we have developed a new colormetric

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epithelium of rat and rabbit and over the esophageal area in the frog lead to a continuous drop which reaches its minimum level of flow at about fifteen to twenty minutes after application and which takes as long as forty-five minutes to recuperate, and in this time the particles still move, but they don't move as fast as they normally do.

Now, I would like to say here that you do not put the irritant on the same area that we afterwards measure so that this is an effect which is slightly removed from this area. The area that is impinged upon where this actual irritant hits is at times completely without activity, nothing is moving there, and it takes a while before movement takes place, but even the rest of the area is effected by the presence of some of these irritants in delaying the movement of the mucus sheath which is secreted by goblet cells in the tracheobronchial tree and which is normally rather rapidly passed along--this is quite a rapid movement--to the direction in which it is most thoroughly eliminated and this type of stagnation we feel is of considerable importance in keeping particulate matter in the animal and in us, for that matter, to be having a metabolical effect.

Next slide, please. Now, we are here on a different topic again and our metabolism story which is going to take the rest of the time, I think.

We were interested in the

method for alkylating agents, epoxides also, and we have done positive results on some of the aliphatic fractions which would seem to indicate that something that alkylates,may be an epoxide is present in some of these fractions.

Any other questions?

DR. ALTSHULLER: Paul Altshuller. I have a comment on these epoxides. Paul Altshuller, Public Health Service, Cincinnati. I don't think we can forget as Dr. Payne pointed out that this is only the organic particulate fraction and there is a excellent probability that many of the lower molecular weight epoxides do not appear in this organic particulate fraction, but in the gas phase;as a matter of fact, there is work on the vapor phase photo oxidation of O's and NO^2 mixtures which was done at Stamford Research Institute in which epoxides were detected and determined in simple systems by means of gas chromatography in the C-4 and C-3 range, and I am sure that the C-5 and C-6 epoxides also present in these mixtures, so whether or not they appear in the particulate fraction, I think there is a likelihood they appear in the gaseous fraction.

CHAIRMAN SAWICKI: Any other questions or comments?

DR. LYONS: Dr. Lyons, New York. As a possible extension of the intratracheal method of bioassay, have you ever considered the work of -- published by Horning a number of years ago --it seems to have been neglected-- in which he disected out the trachial bronchial tree of young mice, mice under two months in age and segmented up the trachia and major bronchi and added carcinogens in crystalized form to these segmented parts and inoculated them into adult hosts of the same strain and produced carcinomas. Now, could you incubate such segmented parts?

DR. PAYNE: We have not tried anything of that sort. We have followed pretty much the routine methods of painting, subcutaneous injection, injection of various sites in the type animal and we are planning in the near futureto expand the development phase of carcinogenic screening and I am sure that Dr. Weisburger who will be coming with us to do some of that will be looking for different methods to try and I am sure will be interested in looing into that as well as others.

CHAIRMAN SAWICKI: Any other questions?

I had one other comment, but I guess I better hold it. It was on the question between skin cancer and lung carcinogenises and I think that takes quite a long time. Maybe we can hold it until after Dr. Falk's paper.

Thank you, Doctor.

Our next speaker is Dr. Hans Falk who obtained his Ph. D. in Biochemistry at McGill

University, Montreal, Canada in 1947. He has been in cancer research since July 1947. One of his first papers was on the pyrolysis of cholesteral at 360 degrees and their relation to carcinogens. I remember we had a seminar at the Cancer Research Laboratory at the University of Florida on this particular paper at that time. He has worked in the etiology of lung cancer environmental agents since 1949. He was at the University of Chicago as an instructor in the Department of Pathology from 1947 to 1952. He is presently at the University of Southern California, Los Angeles, Department of Biochemistry, and I hear he will be moving elsewhere shortly.

He has worked in research on carcinogens in air, auto exhaust, in lungs, etc. He's been a pioneer in the field in this country just like the British workers pioneered in chemical carcinogens and hydrocarbons and air pollution. They really started it going in these fields and Hans Falk has been a pioneer in this particular field. Dr. Hans Falk.

DR. FALK: Thank you. Ladies and gentlemen, I am going to discuss the problems that are facing all of us in evaluating the possibility of conforming the information regarding the micrograms of benzpyrene in the air and the probability of having an effect on human beings, and I have found up to now that I have only a number of reasons to doubt that we will ever be able to really correlate these two group. of data. I am going to present to you the findings so far in four sub-headings: 1. the physical problems that we are facing; the chemical problems, physiological and pathological consideration, and each one technically finishes by itself any easy correlation of the quantitative concentration of air pollutants and their effect.

If I may have the first slide, and no lights. These data are not obtained in our laboratory. They come from Dr. Branch and have been modified on the one slide, and show the time of residence in certain areas in the tracheobronchial tree, the rest of the tissue under consideration, the particle size that is retained at these locations and the quantity of the particle that is retained.

Now, you have lots of information missing on that slide and I am not going to go into that. The main reason is that in the bronchials and in the alveoli most of the type of particle attention is concentrated. We have naturally the retention of particles of practically all sizes and to the greatest extent in the alveoli and to less extent elsewhere. This is actually quite obvious and I just wanted to show it at our starting point for our discussion.

The next slide, please. The next slide shows something quite different, namely the ability of a protein solution to remove from the particulate soot the compound of interest to us and this shows actually that the time required to start elution from soot is rather small. We are having a beginning of no protein solution in forty-eight hours and absolutely no elution of polycyclic takes place, while as soon as protein is present in solution, the various compounds are coming off at a rather similar speed. The smaller molecules seem to have a tendency to be eluted faster than the larger ones on a molecular structure basis.

Next slide, please. On the next slide I wanted to just show a problem that arose during the study of elution, namely the question of whether a molecule of benspyrene which is now eluted by a protein solution is still able to do anything or whether that is now bound to that protein solution in some inexplicable way. The importance of that is that some people believe that such binding is actually occurring very rapidly and this picture is to show that such binding did not occur because these are cells, living cells in tissue culture which have been exposed to a protein solution containing benspyrene for only a short time, and they were afterwards washed with more solution until the background is clear and were then inspected under ultraviolet microscope and pictures were taken after a two minute exposure which showed a considerable amount of fluorescence inside the cells, particularly in certain areas, which I for any better word call vacuoles. They might not be corresponding to the vacuoles that you normally see, but they are distinct areas.

Now, on these pictures you can also see very often the nuclei which are denser and do not seem to have picked up as much of the flucrescent material, so it shows that the benspyrene outside the cell is not bound, but is capable of entering the cell and distributing itself there into concentrated areas, into the general cytoplasmic area and not into the nuclei. At least we have always these dark areas in everyone of those cells.

Now, on the next slide we are entering a problem which I feel is of significance and I have been preaching this particular slide before. When we are trying to analyse the pollution in terms of benspyrene and we are determining how much benspyrene is actually present in the atmosphere, we cannot necessarily say that that much benspyrene is available for carcinogenic action if it were to get to an appropriate site, and the main difference lies in those areas where the particle size becomes around a hundred millimicrons average. It seems that the plasma solution which I feel is a little bit more on the physiological side than the benzene solution gives different results with regard to elution, and it is therefore conceivable that we are getting too high readings for benspyrene after our benzene extraction with or without methanol compared to our plasma extraction of a poor sample of soot, however you get it.

The next slide, please. Another complicating factor was the fact that much of our benzpyrene is kindly contributed to the air by rubber tire dust. It sounds like a very small factor, but it isn't when you just go and compute rubber tire wear, and the fact that one third by rate of the total rubber tire is carbon black. The carbon black used is one of the cheapest that does the job well which is very rich in benzpyrene.

Another way of finding out whether a certain sample is rich in rubber tire dust or just in the ordinary exhaust is to determine the amount of chrysene present and there is much more chrysene in the rubber tire than there is in the automobile exhaust, so that could be used as a distinguishing feature. In any case, however, the rubber tire also gives off benspyrene in the protein solution, although not to the same extent, but I had the impression in the beginning that it would not give up the benspyrene probably at all because the particles are so completely surrounded or caged in by the many materials that go into the rubber tire.

Next slide, please. Now, this is a problem that has been touched upon already before today, and that will be mentioned again in the next few days, and that is the survival of various polycyclic hydrocarbons after emission into the air and there are two different processes at least going on that I can name, one is the oxidated destruction of some of the polycyclic hydrocarbons, and the other one is the disappearance by sublimation of some of the hydrocarbons.

Now, we try to expose them to ordinary atmospheric conditions and then to make things a little better, we give them our artificial smog which helps considerably, and we found that under those conditions we can actually get rid from our sample of all the small particle size -- I mean molecular size now -- compounds. That explains why it is so easy to lose all your napthalene, your phenanthrene, your fluoranthene, your pyrene while you keep most of the larger molecular size compounds intact.

This work was carried on years ago on these compounds on filter paper and in soot. The work on filter paper was much easier. You took the pure hydrocarbon and exposed it to any condition that you wanted to and measured the quantity left over any time.

The work on soot actually was more complicated. You have to take a sample of soot, analyse it before exposure, analyse it again after exposure and try and account for everything quantitative that is left over. However, the data on soot itself was similar to these data here and showed that exposed to atmospheric conditions you lose by sublimation oxidation and other properties, a good deal of the compounds of the 2, 3 and 4 range and you also lose some of the higher ones by oxidation.

Next slide, please. Now, the importance of the previous slide to us was that several of these not found polycyclic hydrocarbons seem to act at the same time as anti-carcinogenic materials under the appropriate conditions and we were therefore very interested to know which ones acted as anti-carcinogens and under what relationship.

Now, in our early studies -- and I am going to show several of them to more or less point out that this is not just a unique situation, but a very common and actually previously described rather common way of showing anti-carcinogenic properties. The compound in question here is dibenzanthracene which I do not consider terribly important in air pollution and reduction products of that compound adding 2, 6 and as many hydrogens that occur. These compounds were prepared by Dr. Lasinski (phonetic) who was sent in to us for our testing and we put them to this use.

Now, it so happened that under these conditions the dihydro and the hexahydro completely prevented the formation of cancers which occurred in the animals without these production products.

On the next slide you see a similar experiment except we changed the vehicle and observed that the vehicle is of considerable importance, although I have not the slightest explanation of how the difference between ethyl cyclo hexane (phonetic) might affect the anti-carcinogenises property of the hexahydro dibenzanthracene.

On the next slide -- I put them all in here. Actually we could have saved a few. They are showing the same principle. This shows again the same compound, the dihydro and hexahydro, but instead of being administered simultaneously they are spaced up to four days apart to see whether the change in this carcinogenic reaction that we always get by having two compounds there was something that had to happen at the same time, simultaneously or whether processes were reversible so that the carcinogenic agent could be displaced by the noncarcinogenic agent at certain intervals of time, and this slide shows that actually in both cases the dihydro and hexahydro, the time of four days was still capable of diversing or preventing the carcinogenic change.

Next slide, please. Now, here we went farther and found out that we can hit the limit for some of these anti-carcinogens at different times. I do not really put a tremendous quantitative importance on these data at the moment because partly they are not quite complete the phenanthrene was injected prior or opposed to the dibenzanthracene. We have a tumor incident of about 65 percent without any phenanthrene and we get pretty much back up to that level by the time we have ~~waited~~ fourteen days after administration of the dibenzanthracene to put in our phenanthrene.

Next slide, please. Here we have compounds that are actually of similar significance because we are now inhibiting the tumorogenic potency of benzpyrene by compounds present in the atmosphere and in ratios that we pick as reasonable ratios appearing in cigarette smoke or auto exhaust or in other products of incomplete combustion, and we find that actually the inhibition was most marked in the case of anthracene, pyrene and phenanthrene at this level.

Now, this was an early time. It was only six months old when this slide was prepared. In the meantime I am older by six months, and so are the mice, and the ratio showed out better because the tumors that we got in the three compounds with only three and four rings have not increased in number where the others in the benzpyrene and some of the others have increased considerably. So the data now looks better than it looked then.

Next slide, please. Now, this slide I mentioned to you before too. It is a different topic. We are dealing here with the automobile exhaust in Los Angeles and the picture is only of significance, I feel, in the and partly because there might be impurities in some of them, so I would just say that by the time you reach fourteen days, and mind you, the minus in front of them means that we put the anti-carcinogen before we put in the carcinogen, and the plus means that we put the anti-carcinogen in after the carcinogen.

In various cases and under various conditions we found that fourteen days before the carcinogen is too long a time to have an effect in the case of the dihydro and something like seven days after the carcinogen is too long a time for the hexahydro, but I wouldn't go as far as to say that we have actually determined the limits in which the carcinogenic actions and counteractions do occur.

Next slide, please. Here we hrve changed to a little more down to earth compound. Phenanthrene was used to set off the not naturally occuring compounds to counteract dibenzanthracene carcinogenises, and we found that under all conditions that we tested we could not ever get beyond a fifty percent inhibition. Consequently the phenanthrene was never an effective as an anti-carcinogen, yet it was still active to some extent at the concentration ratios shown here.

The next slide, please. Here we have the same relationship of phenanthrene to dibenzanthracene. There is variations in the time at which six percent of the hydrocarbons that are emitted into the air being able to give rise to epoxides. It means all the data that we obtained in some research on exhaust gave a tremendously high level of epoxide formation. I did not do that work myself. It was done by some of Institutes for the Air Pollution Foundation and I just used their data without giving them credit for it.

The next slide, please.

Now, this same material caused graciously a smog out there in Los Angeles. It has however other properties which I would like to discuss in a minute. Mainly for the reason that the low cancer in biological testing of material from the Los Angeles area and the predicted incidence of lung cancer today or twenty years from now may have as one of the explanations for the difference. The exposure of an excised trachea and bronchial tree of a rat or rabbit can be used to show that the regular air pollutants in Los Angeles, cigarette smoke, any brand, even the filter type, and the artificial smog that we make ourselves will have identical effect. This effect consists of a stimulation of the movement of particles over the tracheobronchial tree which lasts only about one to two minutes after a stimulation exposure of two to thirty seconds.

Following this period of stimulation you reach a period of inhibition and this inhibition of the movement of particulate matter over the cilliated metabolism of benzpyrene and other polycyclic hydrocarbons for several reasons. One of them was to see whether we could find, for instance, a chemical which appeared to be more carcinogenic than benzpyrene, another one was to find out whether there is potentially any change possible in the normal individual which may lead to a greater retention of benzpyrene so that this change in metabolism may account for greater carcinogenic potency under certain conditions in certain animals and in general to elucidate the effects of material from the outside to the inside in the experimental species.

In these experiments here we were interested in the effect of the vehicle and after subcutaneous injection of radioactive benzpyrene into mice, we followed the elimination in the species and found that the effect of the vehicle was of considerable importance mainly because the rapid elimination in plasma or in an aqueous medium could be contrasted with the slow elimination in a lipid medium and we not quite duplicated, but did similar work in the one described up here using an accelerator we got a curve thatis fairly comparable to an accelerated line of elimination of benzpyrene.

What does that mean in terms of cancer production? In parallel experiments we injected these benzpyrene solutions into the mice and waited our two years and found out that we got the expected tumor yield only in those animals that had been injected with a lipid vehicle, while we got absolutely no tumor where a similar dose was injected in either a protein solution or in an aqueous medium where it was a suspension.

The next slide, please. This is a slide to show the disappearance from the skin of benzpyrene and it might, of course, be said, "Well, you haven't eliminated evaporation of benzpyrene from the skin." We have eliminated all kind of other factors like the licking or scratching or otherwise eating away of benzpyrene by these mice because we had them tranquilized and we had shaved them and they were not able to do anything to their back area while we were measuring the radioactivity and we observed the disappearance during the twenty-four hour period that we observed them.

Now, the next slide we are showing the relationship between the dose per unit area of benzpyrene applied to the skin and the quantity eliminated through the bile in rats that were given radioactive benzpyrene. There is a quantitative relationship in that if you put more on the skin you get more on the bile. It's not too surprising. Also it shows that it takes considerable length of time to get the material back out of these animals.

And the next slide which shows something that looks as odd as it is, namely that when we measure the appearance of benspyrene in the bile, mind you when I say benspyrene in the bile, there is no benspyrene in the bile. This is all metabolized material. This is a collection of twenty-six or more metabolites coming out in the bile of benspyrene and the surprising thing is that it seems to come out in spurts. This is a long period of time, of course, this is an hour; and there are in this particular slide, there are three periods during which time the elimination in the bile at a steady rate of bile flow, higher concentrations are metabolized, are eliminated. I don't know whether the limiting factor is in the skin, in the circulation, in the bile or where it might act, but it gives us a rather peculiar pattern.

The next slide. Well, we are reaching the lung finally and here I am just showing that following the infusion of benspyrene into the trachea of rats depending on whether we are using a plasma solution or an aqueous suspension, we get completely different yeild of biliary metabolite. In the one case they are well, about fifty times as high as in the other, and that was of significance to us particularly in view of the fact that we have later on used mucus instead of protein to see whether the mucus would have a different attack and found that with the addition of mucus to the fluid inside the bronchi, we can practically reduce the elimination of the bile to zero.

Now, I will try to mention that again later.

Next slide. This shows the same data except that here we are showing specific activity of the bile rather than accumulative effect and it again shows that much greater yield of benzpyrene metabolized in the bile after protein solution has been incubated compared to the saline, but it also shows that they both have the same peak which occurs just about at the time that the infusion has stopped and there are several potential explanations for this peak, but I won't go into them because they are quite controversial at the time.

The next slide. After the material has reached the lung it is usually under normal conditions moved by the ciliary apparatus which I described to you a moment ago and reaches the esophagus, it reaches the stomach and goes through the whole intestinal tract to be eliminated, and we wanted to find out whether there were any particular areas in the gastrointestinal tract where adsorption of benzpyrene would occur, and found that the stomach is a rather poor adsorbing medium. We have gotten some adsorption, but it is exceedingly low and it has made no difference whether we use the saline or a protein solution. The adsorption through the mucosa of the stomach is practically nonexistent.

The next slide, please. This shows the adsorption from the duodenum. This narrow shows the considerable difference, again depending on the vehicle used, and the very high adsorption in the protein solution as contrasted with the low adsorption in the saline or accelerator (?).

Next slide. This is just another similar observation on the adsorption from the ascending colon which shows similar quantative data and proves more or less that it depends an awful lot on the medium in which the benzpyrene finds itself whether it is adsorbed or not adsorbed and that is the main reason for showing the slide.

The next slide, please. Here we make a considerable jump. These twenty-eight compounds that have been characterized partly in the bile as products of benzpyrene metabolism contain one major compound which is of significance potentially as a carcinogen and that is the one fraction probably the dihydro monohydroxy benzpyrene glucuronide which is shown here at this area as the 427, 448 that is the fluorescent data compounded at this location on a two-dimensional chromatogram which give rise to benzpyrene on the mildest acid treatment that we have been able to give. Now, this compound would normally be present to the extent of about three percent of the total metabolite of benzpyrene in the bile and consequently in the intestinal tract, and we are interested to know what kind of mild condition would be necessary to change it back to benzpyrene. That this compound exists has been known for quite a long time. It has been mostly described in the literature by Harper and by some other English investigators and we have been able to actually locate the compound, so that it is not just one compound on acid treatment will give rise to the precursor, will give rise to benzpyrene and is thus a precursor of benzpyrene, but we know pretty well what it looks like by the adsorption spectrum, by fluorescent spectrum and by our location.

The compound has been in its analogy described by Dr. Van Duuren for dibenzanthracene and he will possibly talk about that, and it has been found in the case of pyrene and in the case of methyl-cholanthrene and I think 1,2-benzanthracene by Harper, so that the existence of a metabolite that can give rise to an apparent hydrocarbon on very mild treatment might have to be considered as a potential in producing cancer of the colon or somewhere else where this transaction or formation of the hydrocarbon again may take place.

Next slide, please. Here we are now on the final section, the pathological finding on changes in benzpyrene metabolism with which I will conclude the discussion. Here we have treated rats with what we considered just about the right dose of carbontetrachloride for a good effect. The carbontetrachloride is injected subcutaneously into the mice at a dose level of .06 millimeter per hundred gram birth weight and about nine hours after that the liver is seen to be considerably affected . There is a lot of necrosis mycosis and this animal, although it survived this treatment very well, it will show liver damage for three days on the basis of histologic observation. It will show liver damage for three days on the basis of the bromsulphalein test, and it will show liver damage for three to four days on the basis of benzpyrene metabolism, so here we show the total cumulative elimination of benzpyrene after animals have been given carbontetrachloride one to five days prior to the experimental injection of benzpyrene, and we show very little of total dose is eliminated for the first three days while nearly all of it is eliminated as normally in four and five days.

Now, the next slide will show another way of presenting these data, namely the specific activity of the bile shows that the typical very high rate of elimination which normally occurs after about thirty minutes of injection may reach its peak level only after three to four hours and that peak is not a very marked peak. After the peak, the decline is practically non-existent. On the other hand when you reach your fourth day you will see that the actual peak is practically back to where it ought to be and there is still a little delay and elimination which is noticeable on the fourth day. On the fifth day we are actually back to normal. This is the one on the fifth day compared to the control. Now, that means that if we instead of the mice or rat were exposed to a small infection or any kind of minor irritation of the liver which we might not be able to notice and which might not give us enough trouble to either see a doctor or do anything about it, may still be able to retain a fair amount of benspyrene in our system if we were exposed to a fair amount of benspyrene.

Now, under normal conditions eighty percent is eliminated in about six hours and over ninety percent is eliminated in twenty-four hours. Under these conditions we have eliminated only about ten to twenty percent of the total dose in six hours and we would not eliminate the rest of the material in much more time because it is not accumulating as benspyrene or as metabolite anywhere in the organs of the carbontetrachloride treated animal. We do find a certain amount in the liver and a certain amount in the kidney, but these are normal quantities so that the majority, the seventy percent of the total benspyrene that was injected is not really accounted for and it is not going to be eliminated in the next twenty-four hours or so. It just found itself a different place for residence, and I feel that there is a much better chance for a carcinogenic action in such individual where a certain amount of liver damage will help to keep the benzpyrene from being rapidly eliminated.

On the next slide, the next one, please? I am showing the bromsulphalein change, this carbontetrachloride damage, and the first day it starts from the right and goes to the left, there is very low recovery of the bromsulphalein. On the second day the recovery is considerably better. On the third it's as good as normal except on a different slide which I didn't bring along it shows that although the bromsulphalein is now excreted very efficiently it is not conjugated properly, so that most of the bromsulphalein excreted on the third day, although it comes out of the circulation, is not the normal conjugated type, but is free bromsulphalein.

On the next slide it also shows that even though certain metabolites are excreted after the carbontetrachloride damage, the pattern of radioactivity is changed in that you have a new compound or new compounds present here and some of the older ones missing.

Now, on the next slide it is the identical picture except that we are here looking at fluorescent maxima rather than radioactivity. We can actually break it down a little further and see that this compound here is only present after the carbontetrachloride damage to the liver and this one here is absent completely after carbontetrachloride damage; so we have much more information now about what kind of compounds are involved, but I don't think it will make too much difference to the general picture.

I think that's my last slide. Now, lights, please. To just complicate the story a little bit further, I would like to say that we have turned out an experiment by combining two facets which are well known to cause changes in the lung and are both completely independent of benzpyrene and soot, namely, the combination of viruses with smog as we know it in LA. This combination was tested for about three years on mice of the C57 Black strain. These animals were given three injections of influenza viruses which they managed to survive, learned to live with. One group was at the same time given a continuous exposure to artificial smog and this combination of smog and virus infection produced squamous metaplasia in the lung of some of these animals, and produced also a larger group of adenomas in these mice than normally, and it produced sufficient hyperplasia to be a very common occurance.

Now, when we looked at the animals that had only smog, without virus, we did see abnormal production of the lung too, but we did not find either as many or as the pathologists looking at it said as virulent hyperplasia. Now, we have only done that experiment once and there were quite a good number of animals that died without being in any way bothered by either the appearance of adenomas hyperplasia or actual metaplasia, and yet we feel that there is a much more difficult thing to talk away and eliminate because the exposure to these viruses is after all something although not identical, but akin to what we are all exposed to and the smog exposure that these animals were getting was much more than we are exposed to.

The actual concentration of smog that these animals got was at least two or three times as high as the highest exposure that people in the Los Angeles area ever get, and if you realize that the Los Angeles exposure is at the peak exposure, it means that the animals are getting it all day long while the human is getting it from maybe four hours or five hours a day, so the extrapolation of these data to our own experience in LA is not quite justified to consider that an immediate menace. Our smog would have to last longer and be higher and our virus infections would probably have to be more severe on us than they seem to be normally to be able to say this is our fate in LA on further exposure to the same smog and the same viruses that the mice get.

Now, there are a few other points that I hope to put in in the next three minutes and that is that we try to improve on the methods of identifying polycyclic aromatic hydrocarbons and I haven't really touched on that field yet, even though the seminar is mainly on this topic, and I find that a very nice way of getting more information on compounds was to change the actual solvent and determine fluorescent spectra for two different solvents, and if you pick the right solvent, of course, the spectrum can change completely. I found the easiest way of doing it was to change the solvent from something like ether to an aqueous acetic acid solution. This will produce a considerable change, and, for instance, benzpyrene which has a beautiful spectrum in ether will have a practically non-existent spectrum in the green area in aqueous acetic acid.

On the other hand, 11,12-benzofluoranthene, which has a very similar fluorescent spectrum in ether, has pretty much the same fluorescent spectrum in aqueous acetic acid, so this way is one method of differentiating between those two if one is interested in this separation; and another thing on the same line was that one could improve the spectrum, fluorenscent spectrum by changing the temperature at which the spectrum was run from room temperature down to temperature of liquid nitrogen. That usually requires a different instrument than the one that is usually available, but we have one, and Aminco is willing to sell you one I am sure, so that you could run fluorescent spectrum at different temperatures and that again will show changes in intensity of fluorescence and in actual maxima that can be seen. Usually the shift is not anywhere near as great as the shift when you change to an aqueous acetic acid or other solvents. Part of the explanation of the shift, I think, has to do with the solubilization of the compound, as in solution many fluorescent compounds have a different spectrum from suspension and the benspyrene particularly, I think, goes out of solution in this aqueous acetic acid into a very fine micro-crystal and suspension which shows a different fluorescent, and this was just a method that I had divised to be able to be reasonably sure of identification when the readsorption spectroscopy was not coming along because of minute quantities which were only available. So that's about all I wanted to tell you. Thank you very much.

CHAIRMAN SAWICKI: Any questions or comments? Dr. Hoffmann?

DR. HOFFMANN: Hoffmann Sloan Kettering Institute, New York. I have three questions. Question Number 1, you showed in the slide your inhibition study, inhibition of polynuclear aromatic hydrocarbons of carcinogenic reactors to benz(a)pyrene. On how many mice was this experiment done and how great do you estimate the experimental deviation?

DR. FALK: The size of these groups was only thirty animals each, and we have usually found that the total yield of tumors under those conditions that we studied without the inhibitor was only about fifty percent, so we are working there with fifteen tumors in a group and after the inhibition was started we usually ended up with about half as many tumors. Now, the reason that we did not need to go and make statistical analysis of those data and worry about distribution curves was that we had done quite a number of them at the same time so that we had enumerable studies which are similar. We have usually about twenty different groups going at the same time, and for our purposes one could combine quite a number of them because the only variation between them is something like the dose, the time of injection or some other variables like the solvent, and we feel, therefore, that if we always get something off this kind of inhibition that we have enough animals to really be sure that there is such a thing.

Now, I would like to say here at this time that we looked on for other solvents thinking that we might get something that was a better solvent for an inhibition study, and we found one that was worse. We found a vehicle, trimethylene glycol, which has one possibility, namely of disappearing from the carcinogen immediately. Trimethylene glycol is a good solvent for benzpyrene and it is very water soluble, so as soon as it gets into the animal trimethylene glycol has a tendency to disappear, be dissipated all through the tissue, but the benzpyrene comes out in a very fine precipitate. Under these conditions the protective effect, inhibitory effect of other chemicals was absent. It again shows that the solvent is exceedingly important and inhibitory studies have to be carried out with a variety of solvents and afterwards to be interpreted inside our own lungs as to their meaningfulness with regard to the solvents that are present in the body. Does that answer your question?

DR. HOFFMANN: Yes, thank you very much. The second question, you showed a slide in which you described the elimination of C-14 labeled 3,4-benzpyrene -- benz(a)pyrene, metabolite, and you applied this material to the skin of rats. Now, when I recall right, so far nobody succeeded in obtaining this benz(a)pyrene cancer of the skin when you apply it, paint in on the skin of rats, and, therefore, my question is why did you take rats? Was it a special reason?

DR. FALK: Well, what we did there is not that we applied the metabolite of benzpyrene on the rat skin, benzpyrene was applied to the skin of rats as it was applied to every other area that we thought of significance in metabolism. Now, we also did the same work on mice as you saw some of the studies there on the elimination of benzpyrene in the gastrointestinal tract after skin painting on mice which were similar, but in the rat we have one tremendous advantage besides that the animal is bigger and is more easily to operate on them, but the rat does not possess a gallbladder, and the gallbladder in all other species that we had at our disposal ruined our metabolic study because in the gallbladder you have a time during which all the metabolites are exposed to the action of beta nucoronadi Mucuronidase (phonetic) present in high concentration there and which hydrolysis-(phonetic) all are metabolized except those of the sulphate and now when we get this far and try to analyse from metabolites, we get completely fowled up because all of them start to oxidize and disappear on us before we really had a chance to find them. We proved that by using radioactive tracers and we actually found out that the rate of which these things go to pieces after they are free phenyls and hydro-phenyls (phonetic) is fabulous, so after we established that we threw all our mouse studies out and have never presented any metabolic studies on mice any more because they don't mean enough to me.

DR. HOFFMANN: Thank you very much.

CHAIRMAN SAWICKI: Any other questions?

Dr. Lyons?

DR. LYONS: To elucidate a point for Dr. Hoffmann, Shontel and Hays in Glasgow (phonetic) produced, I think, squamous carcinoma on the skin of rats by repeated application of carcinogens over a long period of time.

CHAIRMAN SAWICKI: Any other questions?

DR. THOMAS: Thomas, U.C. This has to

do with the final three minutes of your comment on temperature and fluorescence. You dropped from room temperature down to liquid nitrogen. Beckman is coming out with an instrument in the next three or four months that utilizes the conventional light housing. It's an attachment whereby you don't have to go down to liquid nitrogen. It's the water cooling tubing on there, just dropping ten degrees or twenty degrees centigrade you get a tremendous increase as far as sensitivity and fine structure. I was wondering whether you have ever done any work with temperature outside of the extreme that you took with liquid nitrogen.

DR. FALK: I never did, no. I believe in extremes.

CHAIRMAN SAWICKI: Dr. Hoffmann.

DR. HOFFMANN: There was work in St. Petersburg by the Russian group - - no not Shaubut (phonetic) Decoon (phonetic) had done studies on fluorescent spectra of benz(a)pyrene at different temperatures.

DR. FALK: I might mention that I have seen a fluorescent spectrum of benzpyrene by a French worker who can show there the existence of about twenty-five different peaks maxima and minima and that floored me. I presumed none of our existing machines were anywhere near capable of giving that resolution that this machine has given and if that is really potentially available as an instrument it would solve our problems for identification because by that time we can do away with adsorption spectroscopy, Dr. Lyons, and your fluorescent spectroscopy, but I don't know that this instrument is available. Does anybody know how this information was obtained? I have seen the spectrum, but I have surely never known about existing instruments like that.

CHAIRMAN SAWICKI: Do you have anything to say back there? Mr. Hauser?

MR. HAUSER: It is usually taken with a very high resolution spectrometer like a German three-prism spectrometer, and usually they will be at very low temperature to resolve as many peaks.

DR. FALK: It was at low temperature?

MR. HAUSER: Yes, and there is a recent report in Optics and Spectroscopy of both the fluorescence and the phosphorescence and adsorption with high resolution prism spectrometer. The last issue which is July or August, I believe, Optics and Spectroscopy showing the many bands, many polynuclear hydrocarbons.

CHAIRMAN SAWICKI: Any other questions or comments? All right, that's all. Thank you, Dr. Falk.

I have a few announcements to make. 12:30 tomorrow bring your best smile. They want to take some pictures. They want to take pictures of the group and then some of the more distinguished gentlemen they also want to take their pictures. I don't think I am included, but some of you people are included, and then, of course, at 4:30 we have our rides in front if anybody wants rides, and then we meet tomorrow at 9:00 o'clock. Thank you.

(Whereupon, at 4:30 o'clock p.m. the meeting was adjourned until 9:00 o'clock of the following day.)

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